GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
by
GEORGE H. SHULL
with the cooperation of

WILLIAM E. CASTLE
EDWIN G. CONKLIN
CHARLES B. DAVENPORT
BRADLEY M. DAVIS
EDWARD M. EAST
ROLLINS A. EMERSON
HERBERT S. JENNINGS
THOMAS H. MORGAN
RAYMOND PEARL

Volume 35 JULY, 1950 Number 4

TABLE OF CONTENTS

CONGER, ALAN D., and NORMAN H. GILES, JR., The cytogenetic effect of slow neutrons 397

BRIEGER, F. G., The genetic basis of heterosis in maize 420

BURNHAM, C. R., Chromosome segregation in translocations involving chromosome 6 in maize 446

GERSTEL, D. U., Self-incompatibility studies in Guayule II. Inheritance . . 482

PUBLISHED BIMONTHLY AT MENASHA, WISCONSIN,
BY THE BROOKLYN BOTANIC GARDEN, BROOKLYN 25, NEW YORK, U.S.A.
(Date of issue, July 25, 1950)

EDITORIAL BOARD

CURT STERN, *Managing Editor*, University of California

GEORGE W. BEADLE
California Institute of Technology

WILLIAM E. CASTLE
University of California

EDWIN G. CONKLIN
Princeton University

BRADLEY M. DAVIS
University of Michigan

L. C. DUNN
Columbia University

JOHN W. GOWEN
Iowa State College

DONALD F. JONES
Connecticut Agricultural Experiment Station

M. M. RHOADES
University of Illinois

GEORGE H. SHULL
Princeton University

T. M. SONNEBORN
Indiana University (Representative of the Genetics Society of America)

L. J. STADLER
U. S. Dept. Agriculture, University of Missouri

ALFRED H. STURTEVANT
California Institute of Technology

SEWALL WRIGHT
University of Chicago

Volume 35 JULY, 1950 Number 4

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $6.00 net a year for complete volumes (January-November). Foreign postage, 50 cents additional. Single copies, $1.25 each, postpaid.

As available, volumes 1 to 6 may be had at $12.00 each, and the later volumes at $10.00 each, postpaid. The Business Manager will supply information on request as to volumes and numbers available.

Correspondence concerning editorial matters should be addressed to the EDITOR OF GENETICS, Department of Zoology, University of California, Berkeley 4, Calif.

Business Correspondence, including change of address, etc., should be addressed to 450 Ahnaip St., Menasha, Wisconsin, or BROOKLYN BOTANIC GARDEN, Brooklyn 25, N.Y., U.S.A.

Remittances should be made payable to GENETICS, INC.

Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L. & R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

Genetics

Golden Jubilee Supplement

THE BIRTH OF GENETICS

To commemorate the fifty years' anniversary of Mendelism, GENETICS will publish English translations

of MENDEL'S LETTERS TO NÄGELI

and translations of the first publications, in 1900, of

DE VRIES, H., Sur la loi de disjonction des hybrides. Comptes Rendus **130**: 845-847.

CORRENS, C. G., Mendel's Regel über das Verhalten der Rassenbastarde. Ber. deutsch bot. Ges. **18**: 158-168.

TSCHERMAK, E. VON, Über künstliche Kreuzung bei *Pisum sativum*. Ber. deutsch. bot. Ges. **18**: 232-239.

Mendel's famous letters show the wide range of his biological interest and report on important observations not described in his published papers. In addition they give vivid impressions of Mendel's personality.

The papers by de Vries, Correns, and Tschermak illustrate the different ways in which the three rediscoverers of Mendel's work reached their conclusions and presented their results.

These translations will form a supplement of about 50 pages to the September 1950 issue of GENETICS and will be mailed to subscribers without charge.

The supplement will be of value to a wide circle of biologists, and be suitable for use in classes.

For sale as follows:

Pre-publication Prices (orders to be received before September 1, 1950)	*Regular Prices*
2 copies at 0.85	1 copy $1.00
10 copies at 0.80	10 copies at 0.90
25 copies at 0.70	25 copies at 0.80

Orders should be sent to

BROOKLYN BOTANIC GARDEN, BROOKLYN 25, N.Y.

Please make check payable to GENETICS INC.

Genetics: The First 50 Years

MEETINGS OF THE GENETICS SOCIETY OF AMERICA

COLUMBUS, OHIO

SEPTEMBER 11 TO 14, 1950

The GENETICS SOCIETY OF AMERICA at its annual meeting on December 29, 1949, voted to celebrate in 1950 the 50th birthday of the rediscovery of Mendel's fundamental work. Furthermore, genetics as a science began with the rediscovery of Mendel's original findings, so this proposed celebration may justifiably be called the Golden Jubilee Program of Genetics.

The meetings in 1950 of the Genetics Society are to be held at Columbus, Ohio from September 11 to 14, under the auspices of the American Institute of Biological Sciences. The first day of the meetings of the Society will be given over to the presentation of short papers describing the results of current research.

A general meeting of all the biological societies which will convene at Columbus is scheduled for the evening of Monday, September 11, at which the keynote address on "Fifty Years of Genetics" will be given by R. B. Goldschmidt, Emeritus Professor of Zoology at the University of California. This address will mark the beginning of the Golden Jubilee Program.

The papers to be given on Tuesday, September 12, will include various considerations of "Historical Aspects and the Development of Genetics." On Wednesday morning, September 13, the general topic is "The Physical Basis of the Gene," and that of the afternoon is "The Physiology of the Gene." Three papers on "Cytogenetics" are scheduled for Thursday morning, September 14, to be followed by several dealing with "Genetics and the Food Supply" and "Genetics, Medicine and Man."

The addresses of the President of the American Society of Naturalists and of the President of the American Society of Human Genetics are to be a part of the Golden Jubilee Program.

The final address will be delivered on the evening of Thursday, September 14 by Julian Huxley, until recently Secretary-General of UNESCO, under the title of "Genetics, Evolution and General Thought."

The speakers at the Golden Jubilee Program will survey the development and present status of various phases of genetics. In addition, each speaker is invited to consider probable paths of future thought and discovery. The papers are to be published as a separate volume.

THE CYTOGENETIC EFFECT OF SLOW NEUTRONS*

ALAN D. CONGER AND NORMAN H. GILES, JR.
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Received December 19, 1949

THE study of the biological effect of different radiations has progressed as sources of the various radiations have been developed. The effects of X, gamma, beta and alpha rays, of protons and fast neutrons, and recently of fission fragments have been studied in numerous organisms, both plant and animal. Before the development of the nuclear reactor, however, there was no source of slow neutrons in the amounts necessary for a study of biological effect.

The fast neutrons from uranium fission can be moderated by elastic collision with graphite or heavy water in a nuclear reactor until their velocity is reduced to that of thermal energies (average 2200 meters/sec = 0.025 electron volts); such very slow neutrons are called thermal neutrons. Unlike fast neutrons, whose principal reaction with matter is one of scattering, thermal neutrons because of their very low energy and velocity are more likely to be captured than scattered by the atoms they encounter. Most of the reactions of biological elements with thermal neutrons are capture reactions. After an atom captures a neutron, it forms a new compound nucleus with an excess of energy. This new compound nucleus may then:

1) emit a gamma ray immediately to form a stable isotope, e.g.,[1]

$$H^1 + n \rightarrow [H^2] \rightarrow H^2 + \gamma;$$

2) emit a heavy particle immediately to form a stable isotope, e.g.,

$$B^{10} + n \rightarrow [B^{11}] \rightarrow Li^7 + \alpha;$$

3) emit immediately a capture radiation to form a radioactive daughter which subsequently emits beta or gamma rays at a rate characteristic for the isotope formed, e.g.,

$$N^{14} + n \rightarrow [N^{15}] \rightarrow C^{14*} + p \xrightarrow[6{,}000 \text{ years}]{\text{half life}} N^{14} + \beta^-.$$

It is the immediate or "capture" radiations and the delayed or "decay" radiations which are ionizing and produce the effect; thermal neutrons, of themselves, apparently produce no effect since their kinetic energy is too low to produce recoil nuclei by elastic collisions in matter and since they are uncharged particles.

* This work was done under Contract No. W-7405-eng-26 for the ATOMIC ENERGY COMMISSION, Oak Ridge, Tennessee.

[1] These nuclear reactions are usually given in an abbreviated form much like chemical reactions. The reaction $H^1 + n \rightarrow H^2 + \gamma$ is abbreviated to $H^1(n, \gamma)H^2$.

It appears that the biological effect from thermal neutron exposures would be due to two components of the ionization dose—electromagnetic radiation (gamma rays) and particulate radiation (protons and alpha particles). One of the striking biological effects of radiation is the production of chromosomal aberrations, which has been studied extensively in the plant Tradescantia. It has been known for some time from Tradescantia studies that the yield of chromosomal aberrations is different when electromagnetic and particulate radiations are used (for the same ionization dose, particulate produces more aberrations than electromagnetic radiation). In addition, the yield of "two-hit" or exchange aberrations differs qualitatively for the two types of radiations. With electromagnetic radiation of high intensity, the yield of these exchange aberrations increases as the square of the dose, indicating that each of the two breaks involved in the production of an exchange aberration is produced by a different electron track. With particulate radiation, however, the yield of exchange aberrations increases linearly with dose, indicating that both breaks are produced by a single particle (review in LEA 1947). This background of information on the differences in response to electromagnetic and particulate radiation made Tradescantia an appropriate choice for a study of the qualitative and quantitative biological effect of thermal neutron radiation from the Oak Ridge pile.

PHYSICAL METHODS

Material was exposed to thermal neutrons in a treatment facility especially designed for biological experiments with the object of obtaining the highest intensity of thermal neutrons and the smallest amount of fast neutron and gamma-ray contamination possible. A drawing of this facility is shown in figure 1. The arrangement was in part dictated by other uses of the thermal column. The bottom of the drawing is the position of the uranium-graphite lattice of the pile itself. Above this is an eight-inch layer of bismuth bricks interposed to eliminate the fission gamma rays. Above the bismuth bricks is the thermal column, a seven-foot-tall by six-foot-square column of graphite. In the center of the thermal column and on the center line of the pile is a bismuth "safe"; the central cavity of the bismuth safe is the treatment position. Specimens are inserted and removed from the treatment space by means of a sliding graphite rod with a bismuth plug on the end, to which is fixed a graphite treatment box containing the specimens. When the box is in the treatment position, it is completely surrounded by bismuth. A drawing of the graphite treatment box is shown in figure 2. Specimens were always placed on the "slope" of the box, the lowermost face when the box was in the treatment position. Ventilation of the treatment space is assured by grooves and slots through which air passes in from the outside, down the exit-entry tube into the bismuth safe, and out into the pile through ventilation holes in the safe; the pile is at negative pressure. Temperature inside the safe is room temperature.

Measurements: The method by which a neutron flux (the number of neutrons crossing a square centimeter per second) is measured is based on the fact that when neutrons are captured a radioactive isotope is formed. Other things being

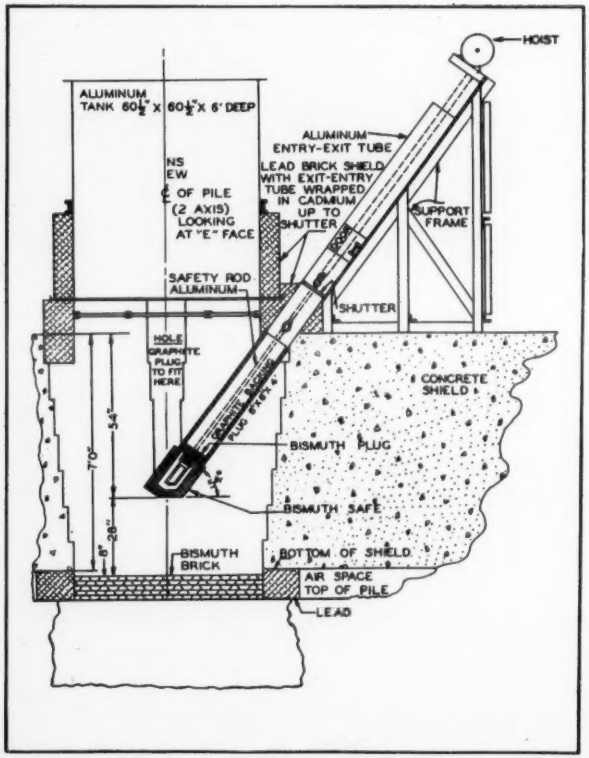


FIGURE 1. Biological thermal neutron treatment chamber.

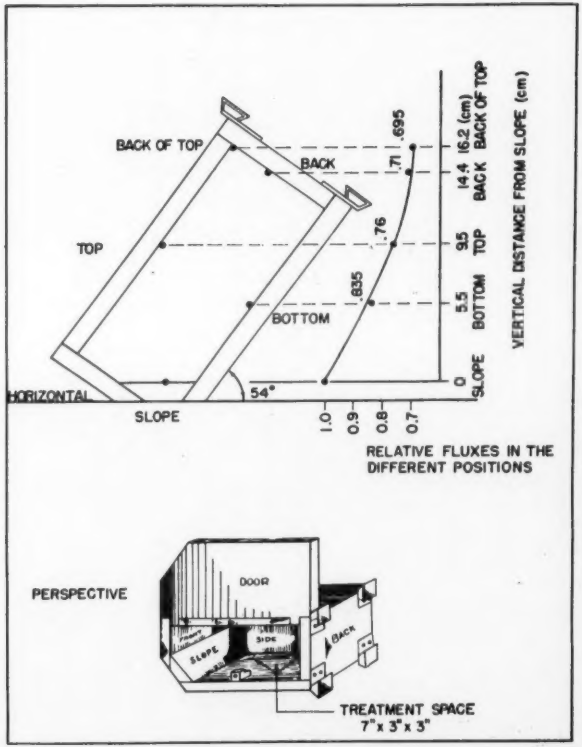


FIGURE 2. Graphite treatment box in the treatment position.

equal, the number of neutrons captured, and therefore the radioactivity of a sample, is proportional to the number of neutrons incident upon the sample, i.e., the flux. The induced radioactivity of the sample can now be measured on an ionization instrument, and this radioactivity measurement is an expression of the flux by which it was activated. The neutron flux was determined from the induced activity in 0.1 cm² indium foils exposed simultaneously and adjacent to the flower buds on the "slope" of the treatment box. Activity of the indium foils was measured as millivolts on a 100 percent geometry ion chamber (JONES and OVERMAN 1948) and from this the neutron intensity, in the arbitrary unit of millivolts of saturated activity per gram of indium, could be determined.[2] The standard error (S.E.) of these measurements varied from 0.5 to 2 percent of the mean value.

Conversion of the arbitrary unit of neutron intensity into the absolute unit, thermal neutrons per cm² per second, was made by three independent methods from:

1) a comparison of the activity induced in the indium foils in the Oak Ridge standard pile[3] (at a known thermal neutron flux) with the activity induced in the thermal column. All measurements were made on the same ion chamber; the value determined is an average of nine separate foil activations.
2) a comparison of the beta activity induced in gold foils in the Oak Ridge thermal column with the activity induced in the same foils exposed to a known thermal neutron flux (by beta-gamma coincidence techniques) in the Argonne National Laboratory pile. These foils were activated and calibrated by DR. AL WATTENBERG of the Argonne Laboratory. All

[2] The formula used to obtain the intensity is: Saturated activity per gram of foil→millivolts

$$= \frac{\text{count}_{(mv)}}{\text{wt (activation factor)(decay factor)}} = \frac{C}{w(1 - e^{-\lambda t_a})(e^{-\lambda t_d})}$$

λ = decay constant
t_a = time of activation = time of exposure
t_d = time of decay, from removal to measurement

and since

$$\text{Dose} = \text{flux} \times \text{time} = \frac{\text{neutrons}}{\text{cm}^2\ \text{sec}} \times \text{sec} = \frac{\text{neutrons}}{\text{cm}^2}$$

then

$$\text{Dose} = \frac{CKt_a}{w(1 - e^{-\lambda t_a})(e^{-\lambda t_d})}$$

K = Conversion of mv/gm→neutrons/cm² sec

For cases where the time of exposure is small in relation to the half life, the integrated dose as measured by the foil can be calculated directly from:

$$D = \frac{CK}{w(e^{-\lambda t_d})\lambda}.$$

[3] The standard pile is a large mass of graphite containing a radium-beryllium source of neutrons. The flux of thermal neutrons at various positions in this pile had been calculated by the application of neutron diffusion theory and a knowledge of the Ra-Be source strength.

measurements were made on the same Geiger-Müller beta counter, the value is an average of four different foil activations.

3) measurements of the activity induced in cobalt samples made on the ion chamber which had been calibrated for absolute measurement of Co^{60} disintegrations per second; the value is an average of seven activations.

The results of these three methods are given in table 1.

TABLE 1

Determination of absolute thermal neutron flux (n_{th} per cm^2 per sec) *on the "slope" of the graphite treatment box in the thermal column. Pile constant at full operating power.*

METHOD	THERMAL NEUTRON FLUX (n_{th} per cm^2 per sec)
1. Indium foils, 4 mil, 25 cm^2. Comparison of standard pile-thermal column activations.	1.09×10^9
2. Gold foils, 4 mil, 1 cm^2. Comparison of Argonne thermal column—Oak Ridge thermal column.	1.29×10^9
3. Cobalt—wire and Co_3O_4 powder measured against known standards.	1.31×10^9
4. Accepted value.	1.25×10^9

Of the three methods, the largest number of measurements was made on indium foils. The accepted value represents quite well the data presented in the table, and will be used as the flux in all subsequent calculations.

As explained above, the neutron intensity to which the plants were subjected can be expressed accurately (±2 percent) in the arbitrary unit of millivolts (per gram indium activity at saturation). The conversion of this arbitrary unit to the absolute units of n_{th}/cm^2 sec is subject to some error and possible revision, so the method of conversion is given here:

a flux of $1.25\times10^9 n_{th}/cm^2$ sec = 89,650 mv/gm satd. activ. of indium foils measured at the 10^9 resistance on the ion chamber, so

$$1 \text{ mv (at } 10^9 \text{ resist.)} = \frac{1.25 \times 10^9}{89{,}650 \text{ mv}} = 1.394 \times 10^4 \text{ } n_{th}/cm^2 \text{ sec.}$$

This value has been used in all conversions of millivolts into neutron flux.

A summary of the physical characteristics of the graphite treatment chamber is given in table 2.

From the data in table 2, it can be seen that practically none of the radiation received by a specimen in the treatment box is due to fast neutrons; roughly, only $1/8\times10^4$ of the activity induced by this neutron field is due to neutrons with an energy >0.3 electron volts.

Measurement of the inherent gamma-ray contamination inside the facility has been one of the difficult problems of this experiment, since any device put into the bismuth enclosure to measure gamma radiation itself captures neu-

trons and may emit its own gamma rays. Two different methods of estimating the gamma-ray contamination have been tried.

1) Using data on the neutron flux and the gamma-ray intensity at the top of the thermal column, the expected gamma-ray intensity at the position of the bismuth safe would be in the ratio of the fluxes. The attenuation of this gamma-ray intensity in going through the 7.94-cm walls of the bismuth safe has been calculated using the absorption coefficient ($\tau+\sigma_a+K$ but not including σs) for lead corrected for the ratio of bismuth/lead density. The calculation using the values described shows that with the pile operating at full power, the inherent gamma-ray contamination inside the bismuth safe is ~6 r/minute.
2) The second method[4] was to expose in the safe a Victoreen chamber enclosed in a boron[10] coated aluminum can; the boron transmitted the inherent gamma rays but absorbed nearly all the neutrons, emitting its own 0.38 mev capture gamma rays as a result. The attenuation of gamma ray intensity caused by interposing increasing thicknesses of lead between the Victoreen chamber and the boron gave a compound absorption curve; the extrapolation of the energetic gamma ray curve to the zero ordinate showed the inherent gamma ray level to be ~5 r/minute.

These two estimates of the inherent gamma-ray contamination in the treatment position with the pile operating at full power indicate that it is about 6r/minute or 5 r/minute. The value of 6 r/minute will be accepted as the proper one though it is felt that this is a maximum value and the true value is probably somewhat lower.

TABLE 2

Physical characteristics of the "slope" of the graphite treatment box. Pile constant at full power.

CHARACTERISTIC	VALUE	SOURCE
1. Temperature	room temp.	by air circulation drawn from outside into the pile
2. Size of box, inside dimensions	17.5×7.5×7.5 (cm)	figure 2
3. Neutron flux	1.25×10^9 n_{th}/cm^2 per sec	table 1
4. Fast neutron contamination	insignificant	cadmium ratio* of indium foils 8.3×10^4, av. of 3 meas.
5. Inherent gamma-ray contamination	~6 r/min	see text, and table 9
6. Relative flux at different positions in box	see figure 2	simultaneous activation of 0.1 cm^3 indium foils placed at the different positions
7. Flux at different positions on the "slope"	uniform	(same as 6. above)

* cadmium ratio is the ratio of the activities induced in a bare indium foil and in a cadmium covered indium foil, and is roughly equal to:

$$\frac{\text{total activity due to neutrons of all energies}}{\text{activity due to neutrons of } > 0.3 \text{ ev only}} \rightarrow \frac{\text{slow + fast neutrons}}{\text{fast neutrons}}.$$

[4] This method was developed and the measurements made by G. E. STAPLETON of this laboratory.

BIOLOGICAL METHODS AND MEASUREMENTS

Inflorescences of a clonal line (Sax no. 5) of *Tradescantia paludosa* Anderson and Woodson were used for all experiments. The plants had been grown in pots for about a year before use. Inflorescences were picked the morning of use, stripped of leaves and bracts and made up into groups of about 12 inflorescences arranged flat so there was no overlap of the buds and held together with a 3–4 mm-wide strip of scotch tape near the butt end of the stems. Each group of inflorescences was carefully positioned so that the buds were lined up on the center of the slope of the treatment box. A 0.1 cm^2 indium foil was placed on the slope of the box about 0.5–1 cm away from the buds. The flux at different positions on the slope had been checked previously and found to be uniform so that all inflorescences received the same dose of thermal neutrons. The treatment box containing the inflorescences and the foil was run into the thermal column for varying lengths of time and the amount of neutron radiation received determined from the measured activity of the simultaneously exposed indium foil.

Aceto-carmine smears were made of the anthers of the treated inflorescences 22–24 hours and four days after radiation and the frequency of the various types of chromatid and chromosome aberrations scored from the temporary slides. Slides were scored under code numbers to prevent bias in scoring. All scoring of aberrations in these experiments was done by one person to eliminate further any bias or difference there may have been in method of scoring. The terminology of aberration types is that of SAX (1940) for chromosome aberrations and of CATCHESIDE, LEA and THODAY (1946) for chromatid aberrations.

RESULTS

The first series of experiments, numbers 9, 10 and 11, were made to analyze the frequency of chromosome aberrations observed four days after irradiation. The data from these three experiments are presented in table 3. Neutron intensity and neutron dose are given in both arbitrary units (mv) and absolute units (n_{th}/cm^2) as described previously using the conversion factor given. Aberrations are as described above.

The data of table 3 are presented graphically in figure 3 following the subsequent tables. A consideration of these and the subsequent biological results will be made in the discussion.

The time required to produce an appreciable number of chromosomal aberrations proved to be quite long—up to 28 minutes for the longest exposure—and the chromosomal aberrations were therefore being produced at a rather low rate. It has been shown in this material (SAX and SWANSON 1941) that the yield of aberrations from cells irradiated in prophase (and which will be in metaphase of division about 22–24 hours later) is three to three and a half times as great as the yield from cells radiated in the resting stage (which will be in metaphase four to five days later). Accordingly, two experiments (numbers 12 and 13) were made for the analysis of the 22–24-hour chromatid aberrations, the longest exposure requiring only eight minutes; the 22–24-hour chromatid aberrations were therefore being produced at a much higher rate

TABLE 3

Frequency of four-day chromosome aberrations from thermal neutron radiation. (See figure 3.)

EXPERIMENT	TREAT.	EXPOSURE TIME SEC.	INTENSITY* MV. (AT 10^9 RESISTANCE)	DOSE		NO. OF BUDS	NO. OF CELLS	FRACTION NORMAL CELLS	ABERRATIONS			
				MV. SEC. (ARBITRARY) $\times 10^{-6}$	n_{th}/cm^2† (ABSOLUTE) $\times 10^{-11}$				EXCHANGES‡		DELETIONS§	
									NO.	PER CELL ± S.E.	NO.	PER CELL ± S.E.
No. 9	A	290	101,000	29.4	4.10	4	379	0.83	31	0.0818 ± .0091	39	0.103 ± .0069
(18 Aug.	B	580	101,000 ± .5%	58.4	8.15	5	311	.73	46	.148 ± .021	57	.183 ± .026
1948)	C	1160	78,600 ± .3%	91.2	12.7	5	474	.59	110	.232 ± .022	161	.339 ± .029
No. 10	B	840	97,200 ± .6%	81.6	11.4	4	710	.54	166	.234 ± .018	268	.374 ± .023
(3 Sept.	C	1680	96,800 ± .2%	163	22.7	4	830	.34	416	.502 ± .022	476	.574 ± .027
1948)												
No. 11	A	372	94,500 ± .4%	35.2	4.91	10	1,666	.77	144	.0864 ± .007	304	.183 ± .011
(14 Oct.	B	744	93,200 ± .2%	69.4	9.67	11	1,038	.61	198	.191 ± .014	328	.316 ± .018
1948)	C	1116	90,200 ± .8%	101	14.0	8	513	.47	144	.281 ± .028	211	.411 ± .030
	D	1488	89,100 ± 1.0%	133	18.5	7	543	.35	198	.365 ± .024	357	.657 ± .012

* Neutron intensity in arbitrary unit + S.E.

† mv. sec. $\times 1.394 \times 10^4 = n_{th}/cm^2$, see text.

‡ Exchanges = dicentrics + centric rings + 2(tricentrics) ± S.E. (where population size = no. of cells scored)

§ Deletions = terminal + large and small isodiametrics (but excluding acentric rings)

than the four-day chromosome aberrations. The data from experiments 12 and 13 are given in table 4, and these results are presented graphically in figure 4.

An X-ray experiment was made to compare with the results of aberration analysis from thermal neutron experiments 12 and 13. Since it was desired

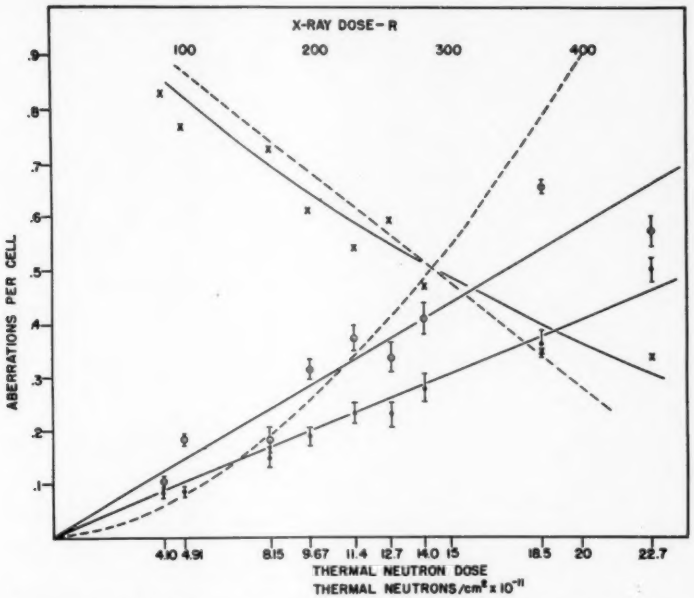


FIGURE 3. Yield of four-day chromosome aberrations from thermal neutron radiation. Data in table 3.

——— Thermal neutrons

- - - - X-rays

● chromosome exchanges = dicentrics + centric rings + 2(tricentrics)

○ chromosome deletions = terminal + large and small isodiametrics

× fraction of normal cells = no aberrations

Lines drawn are the least squares fit to the data.

Curves for the chromosome exchanges and fractions of normal cells resulting from X-radiation (average 50 r/min) are superimposed for comparison.

X-ray curves were made from data taken from: SAX. K. 1948, Oak Ridge Information Meetings; MARINELLI, NEBEL *et al.*, 1942. Amer. J. Bot. 29: 866–874; GILES, N. H., JR. and H. P. RILEY, 1949. Proc. Nat. Acad. Sci. **35**: 640–646.

that the thermal neutron and X-ray exposures be given at approximately the same intensity, X-rays were given at a rate calculated to produce the same number of aberrations in the same exposure time. X-rays were given at 250 kv peak, 15 ma with inherent filtration equivalent to 3 mm Al and added filters 1/2 mm Cu and 1 mm Al. Dose was measured with a Victoreen chamber calibrated sometime previously by the Bureau of Standards. Measurements

TABLE 4

Frequency of 22–24-hour chromatid aberrations from thermal neutron radiation. (See figure 4.)

EXPERIMENT	TREAT.	EXPOSURE TIME SEC.	INTENSITY MV. (AT 10^9 RESISTANCE)	DOSE		NO. OF BUDS	NO. OF CELLS	FRACTION NORMAL CELLS	ABERRATIONS							
				MV. SEC. (ARBITRARY) $\times 10^{-6}$	n_{th}/cm^2 (ABSOLUTE) $\times 10^{-11}$				CHROMA. DELET.	ISOCHROMATID DELETIONS		CHROMATID + ISOCHROMATID		EXCHANGES*	
									NO.	NO.	PER CELL ± S.E.	NO.	PER CELL ± S.E.	NO.	PER CELL ± S.E.
No. 12	A	120	95,000 ± 3%	11.4	1.59	9	713	0.58	190	132	0.185 ± .017	322	0.452 ± .027	53	0.074 ± .011
(9 Dec.	B	240	93,300 ± 1.6%	22.4	3.12	3	401	.35	161	177	.442 ± .033	338	.843 ± .046	61	.152 ± .021
1948)	C	480	97,600 ± .5%	46.9	6.53	5	666	.14	470	452	.679 ± .032	922	1.39 ± .045	200	.301 ± .023
No. 13	A	180	92,400 ± 3.6%	16.6	2.32	5	420	.48	171	99	.236 ± .025	270	.658 ± .036	39	.093 ± .015
(12 Jan.	B	360	93,800 ± .6%	33.8	4.71	4	300	.28	162	139	.463 ± .035	301	1.00 ± .037	51	.170 ± .025
1949)															

* Exchanges = c/c symmetrical + asymetrical interchanges (CATCHESIDE).
= chromatid dicentrics + exchanges (SAX).

TABLE 5

Frequency of 22–24-hour chromatid aberrations from X radiation. Average intensity, 17 r/min. 250 kv peak, 15 ma; equiv. 3 mm. Al. inherent, added ½ mm Cu, 1 mm Al.

EXPERIMENT	TREAT.	EXPOSURE TIME (sec.)	INTENSITY r/min.	DOSE r	NO. OF BUDS	NO. OF CELLS	FRACTION NORMAL CELLS	ABERRATIONS						
								CHROMA. DELET. NO.	ISOCHROMATID DELETIONS		CHROMATID + ISOCHROMATID		EXCHANGES	
									NO.	PER CELL ± S.E.	NO.	PER CELL ± S.E.	NO.	PER CELL ± S.E.
X Ray	A	86	17.6	25.2	3	795	0.78	141	50	.0629 ± 0.0088	191	.240 ± .017	13	.0163 ± .0045
No. 14	B	172	17.6	50.4	3	450	.62	126	58	.129 ± .013	184	.409 ± .032	24	.0533 ± .011
(24 Jan.	C	344	16.1	92.2	6	400	.40	191	135	.338 ± .030	326	.815 ± .062	49	.123 ± .018
1949)	D	585	16.3	159	4	260	.10	291	170	.654 ± .044	461	1.77 ± .069	69	.266 ± .033

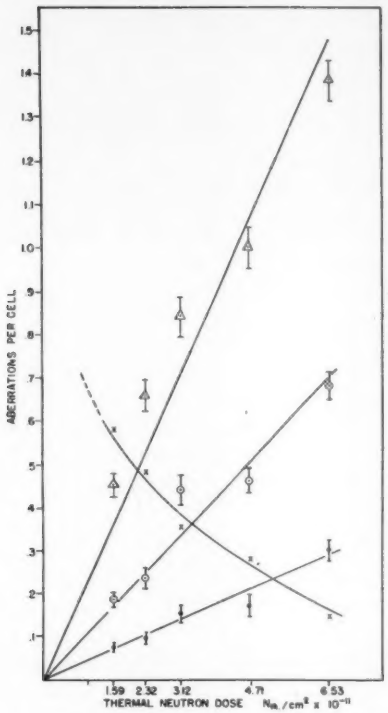


FIGURE 4. Yield of 22–24-hour chromatid aberrations from thermal neutron radiation. Data in table 4.
● chromatid exchanges = c/c interchanges
○ isochromatid deletions
△ chromatid + isochromatid deletions
× fraction of normal cells = no aberrations

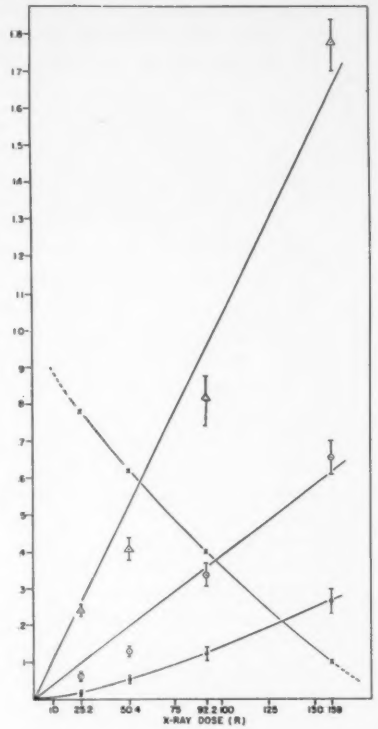


FIGURE 5. Yield of 22–24-hour chromatid aberrations from X-radiation. Average 17 r/min. Data in table 5.
● chromatid exchanges = c/c interchanges
○ isochromatid deletions
△ chromatid + isochromatid deletions
× fraction of normal cells = no aberrations

were made before, during and after each treatment of inflorescences. Absolute exposures may be in error by ± five percent, relative exposures are more accurate. The data of the X-ray experiment, X-14 are given in table 5, these results graphically in figure 5.

DISCUSSION

Before we can discuss the results just presented, we must consider the radiations to which the material has been exposed. The ionization produced in the Tradescantia tissue in the present experimental device can be analyzed into the following components:

1) Contamination:
   a) fast neutron contamination

b) inherent gamma-ray contamination

2) From thermal neutron capture reactions in the inflorescences:
   a) from immediate "capture" radiations, of which the most important are due to:
      1) hydrogen
      2) nitrogen
      3) boron
   b) from subsequent radioactive decay from the radioactive isotopes formed.

These components of the ionization dose will be considered in order. The levels of inherent fast neutron contamination (zero) and gamma-ray contamination (about 6 r/minute, max.) have been indicated already in table 2.

Let us consider the kinds and amounts of ionizing radiations produced by thermal neutron capture reactions. Since these reactions are ones of capture by the atoms of the elements present in the tissue, the kinds of ionizing radiations emitted will depend on the emission reactions of the elements, which are known; the amount emitted by any element, and the amount absorbed in the tissue will depend on the values of the following characteristics:

Ionization energy per unit volume of tissue per unit time =

$$F \times N \times \sigma \times E \times A \qquad (1)$$

where:

1) F = thermal neutron flux = number of neutrons crossing a square centimeter per second, which is the same for all elements.
   $= n_{th}/cm^2$ sec
2) N = the number of atoms of an element per unit volume of tissue

$$= \frac{\%\text{ of wt.} \times 0.602 \times 10^{24}}{100 \times \text{at. wt.}} = \text{atoms/cc. Density} = 1$$

3) σ = capture cross section of an element for thermal neutrons, which is the area in cm^2 that a single nucleus of an element presents to a beam of incident neutrons.
   = value in "barns" $\times 10^{-24}$ cm^2
4) E = energy of the radiation(s) emitted by the element after a neutron is captured.
   = mev (million electron volts)
5) A = the fraction of this energy E emitted per reaction which is absorbed in the tissue; this depends on the type of radiation (α, β, or γ), its absorption coefficient in the tissue, and the thickness of tissue to be traversed.
   $= 1 - e^{-\mu t G}$ where:
      μ = abs. coeff. in cm^{-1} for the radiation in tissue
      t = thickness of tissue, cm
        = .16 cm, radius of the whorl of six anthers
      G = geometry correction for radiations losing only a small fraction

of their energy in the tissue; it decreases the thickness of tissue traversed by the factor 0.75, as will be explained later.

Expressing all the values of equation (1) in the proper units and using the proper conversion values of mev into roentgen-equivalent-physical (rep), a good estimate of the amount of ionization produced in a given volume of tissue by any element for a given flux of thermal neutrons will be:

$$\text{rep per minute} = \frac{(F \times 60)N\sigma(E \times 10^6)A}{5.22 \times 10^{13}} \qquad (2)$$

where 1 r = 83 ergs/gm tissue

$$1 \text{ e.v.} = 1.59 \times 10^{-12} \text{ ergs}$$

hence

$$1 \text{ r} = \frac{83}{1.59 \times 10^{-12}} = 5.22 \times 10^{13} \text{ e.v.}$$

Calculations of the rep/minute due to each of the elements present in the Tradescantia tissue have been made using equation (2); the results are given

TABLE 6

rep/minute of ionization absorbed in Tradescantia tissue due to capture reactions of each of the elements present. Constant flux of 1.25×10⁹ n_{th}/cm² per sec. Discussion in text; see Equation (2).

ELEMENT	ATOMIC WEIGHT	PERCENT* OF WET WEIGHT	N NO. OF ATOMS PER CC. ×10⁻²⁴	σ CAPTURE CROSS SECTION ×10²⁴ cm²	E CAPTURE RADIATION EMITTED AND ENERGY		A FRACTION OF ENERGY ABSORBED IN TISSUE	REP/MIN. ABSORBED IN TISSUE	PERCENT OF TOTAL REP/MIN.
					TYPE	ENERGY (mev)			
H	1.008	10.5	.062,6	0.32	γ	2.23	.003,1	0.194	16.0
B	10.82	.000,287	.000,000,16	715.	α^{++}	2.40	1.0	.394	32.3
C	12.01	6.45	.003,23	.004,5	γ	5.0	.002,6	.000,28	.02
N	14.008	.997	.004,29	1.7	p^+	0.6	1.0	.628	51.5
O	16.00	81.3	.030,6	<.001	γ	>2	.003,1	.000,27	.02
Na	22.997	.100	.000,026	.45	γ	>1	.003,9	.000,07	.005
Mg	24.32	.040	.000,009,9	.3	γ	>1	.003,9	.000,017	.001
P	30.98	.127	.000,024,7	.23	γ	>1	.003,9	.000,32	.03
S	32.06	.066	.000,012,4	.4	γ	>1	.003,9	.000,028	.002
Cl	35.457	.032	.000,005,44	40.	γ	>2.8	.003,2	.000,28	.02
K	39.096	.35	.000,053,9	2.5	γ	>1	.003,9	.000,75	.06
Ca	40.08	.01	.001,001,5	.5	γ	>1	.003,9	.000,004	.000,3

* Chemical analysis: H, C, N, O, P, S, Cl
Spectrographic analysis B = 2.87 ± .06 ppm.
LEA, '47, page 7, Na, Mg, Ca. K

in table 6. Table 6 shows that hydrogen, nitrogen, and boron reactions account for 99 percent of all the ionization produced in the tissue by neutron capture, and that the ionization produced by all the other elements combined is insignificant.

The chemical composition of the tissue was determined from anther samples taken from the same plants and from buds at about the same stage as those

irradiated. The major elements were analyzed for chemically[5] from a sample of ~1,200 anthers. A second sample (~2,200 anthers) was analyzed spectrographically for the minor elements,[6] since some of these might be important in capture reactions because of their extremely high cross sections; examples are cadmium, gadolinium, etc. The spectrographic analysis, shown in table 7, demonstrated that all the minor elements, except boron, could be ignored in these considerations.

Capture cross sections are from WAY and HAINES (1948) and are the most recent and best values.

TABLE 7

Spectrographic analysis of Tradescantia anther ash. 2.2-gm sample, ~2,200 anthers. (For chemical analysis, see table 6.)

ANALYSIS	ELEMENTS
Very strong	Ca
Strong	Mg
Moderate	Al, Fe, K, Mn, P
Weak	Cr, Na, Si
Very weak	Cu
Trace	Ni
Faint trace	B(2.87 ± .06 p.p.m. wet wt.) Mo, Pb, Sn, Ti?
Very faint trace	Ag
Sought, not found	As, Au, Ba, Bi, Cd, Co, Dy, Er, Eu, Gd, Ge, Hf, Ho, In, Ir, La, Li, Lu, Nd, Pr, Pt, Rb, Re, Rh, Ru, Sb, Sc, Sm, Sr, Tb, Tm, V, Y, Yb, Zr

The values for the radiation emitted and its energy are taken from various sources in the literature. The radiations in table 6 are only the "capture" emissions from the compound nucleus, and are emitted almost instantaneously after capture; the radiations emitted subsequently by whatever radioactive daughter elements that may be formed are not considered.

The fraction of the energy emitted which is absorbed in the tissue is important only in the case of gamma-ray emission, since the paths of the 0.63-mev nitrogen proton and the 2.4-mev boron alpha particle are so short in tissue (~10μ for the proton and ~13.5μ for the alpha particle) that all the energy of these particles will be absorbed as ionization produced within the tissue. Only a very small fraction of the gamma rays' energy will be expended in producing ionization within the tissue however, and this fraction can be estimated as has been shown.

A geometry correction (0.75) of the loss of gamma-ray energy is necessary because the gamma rays can originate anywhere on a radius of the whorl of anthers in the bud but will have a biological effect only by the ionization produced within the anthers. An integration of the distribution of reactions shows that the average gamma ray will traverse 0.75 times the radius of the whorl of anthers.

[5] The quantitative analysis was made by the OAKWOLD LABORATORIES, Alexandria, Virginia.
[6] The spectrographic analysis was made by CYRUS FELDMAN of this laboratory.

The last source of ionization within the tissue, that due to the induced radioactivity from neutron capture, is still to be considered. Hydrogen and boron, which account for most of the capture processes in tissue, have no subsequent radioactivity and can be ignored. Considering all the elements present in the tissue and using the values for percent of wet weight and the cross sections, it was calculated that of all the capture reactions that occurred in the tissue, hydrogen accounted for 72 percent, nitrogen for 26 percent, chlorine for 0.8 percent, boron for 0.4 percent and potassium for about 0.5 percent; no other element accounted for as much as 0.1 percent. It can be shown from the data in table 6 and from other considerations of half life and types of radiations emitted that the only element which could contribute appreciably to the total ionization dose absorbed by reason of its radioactive decay is chlorine^{38}. Chlorine^{38} decay is as follows:

$$\beta^- \text{ 4.9 mev (53\%), 2.8 mev (11\%), 1.2 mev (36\%)}$$

$$\gamma \text{ 1.60 mev, 2.15 mev.}$$

and the total amount of absorbed ionization due to chlorine decay can be estimated as has been done previously for the other elements. The greatest uncertainty exists in the estimate of the fraction of the beta ray energy absorbed, in which conditions of geometry, chlorine distribution and other factors can lead to considerable differences in the result. Using two different methods of

TABLE 8

Components of the ionization dose absorbed in 1 gram (1 cc) of Tradescantia tissue, at constant flux of 1.25×10^9 n_{th}/cm^2 sec. Data from previous tables and calculation.

SOURCE OF COMPONENT	TYPE OF RADIATION	rep/MIN	rep PER 1×10^{11} n_{th}/cm^2	PERCENT OF TOTAL rep	PERCENT OF CAPTURE rep
(1) Fast neutron contamination	p^+ recoil	negligible	negligible	—	—
(2) Inherent gamma-ray contamination	γ	6.0 r max.	8.0	82	—
Capture processes					
(3) H capture	γ	0.195	.260	2.7	15
(4) N capture	p^+	.628	.837	8.6	48
(5) B capture	α^{++}	.394	.525	5.4	29
(6) Induced radioactivity (from Cl^{38})	β^-, γ	~.100 (.52 max)	.133 (.69 max)	1.4	7.5
Subtotals: all capture: H+N+B+Cl	$\gamma+p^++\alpha^{++}+\beta^-+\gamma$	—	1.75	18.0	—
from N+B only	$p^++\alpha^{++}$	—	1.36	14	78
Total: γ contam.+capture	γ, $+\gamma+p^++\alpha^{++}+\beta^-+\gamma$	—	9.75		

estimate, the limits of the total ionization absorbed from chlorine^{38} decay ($\beta^- + \gamma$) is ~0.10 rep/minute, but could extend up to a maximum of 0.52 rep/minute. To check on the induced radioactivity, a sample of buds was counted for four hours after removal from a 30 minute exposure and found to decay with a 37 minute half life, corresponding to the decay of chlorine^{38}. The C^{14*} 0.15 mev beta ray could not be detected by the method used, but at worst could amount to only 0.003 rep in two days, an insignificant amount. We conclude that the contribution to the ionization dose from radioactive decay is small and is almost entirely due to the decay of chlorine^{38}.

Using all the data which have been presented previously, it is now possible to express the sources and amounts of the ionization energy which are expended in the Tradescantia tissue; this is done in table 8. The unit of dose is $1 \times 10^{11} n_{th}/cm^2$ as this is the unit dose in the biological experiments. It is seen that the gamma ray contamination makes up a large share of the rep absorbed; these are physical values, however, and do not involve the relative biological efficiencies of the various types of radiations, which will be considered later.

Thermal neutron and X-ray biological equivalence and efficiencies: The data given in the section on biological results are a quantitative measure of the identical response produced by thermal neutrons and X-rays. All possible care was exercised to be sure that the only difference between the X-ray and thermal neutron experiments was the radiation itself. Therefore, the dose of radiation, X-ray and thermal neutron, required to produce the same amount of effect is a measure of the biological equivalence of thermal neutrons to X-rays. This neutron/X-ray comparison has been made for three classes of aberrations: isochromatid deletions, chromatid plus isochromatid deletions, and fraction of normal cells; the equivalence based on isochromatid deletions is probably the most accurate one, since these aberrations can be observed more accurately than the other types. These "one-hit" aberrations can be compared because it has been repeatedly demonstrated (LEA 1947; KOTVAL and GRAY 1947) that they vary linearly with dose for all radiations, X, gamma, and heavy particles (the same is not true for the exchange or "two-hit" aberrations).

A correction must be made in the thermal neutron experiments for the fact that a certain proportion of the ionization dose is due to the inherent gamma-ray contamination of the facility and is not due to thermal neutrons at all (table 8); the same correction applies to the amount of biological effect observed, which is due to thermal neutrons plus inherent gamma-ray contamination (figs. 3 and 4). The correction has been made by subtracting from the total effect due to neutrons plus gamma rays, the amount of effect due to that dose of gamma rays (8r) associated with a unit dose of thermal neutrons ($1 \times 10^{11}/cm^2$), as follows:

$$E_{n\ alone} = (E_{n+\gamma}) - E_\gamma$$

where:

E_n = effect due to dose D_n of neutrons

E_γ = effect due to dose D_γ of gamma rays associated with D_n of neutrons

$E_{n+\gamma}$ = effect due to $D_n + D_\gamma$.

TABLE 9

Biological equivalence and efficiency of thermal neutrons/X rays. Data from previous tables and figures.

BIOLOGICAL EFFECT	COEFFICIENT OF PRODUCTION PER CELL = C			BIOLOGICAL EQUIVALENCE‡		DAILY "TOLERANCE DOSE" 8 HOURS	REP.§ FROM 1×10^{11} n_{th}/cm^2	BIOLOGICAL EFFICIENCY‖	
	$n_{th}+\gamma$ CONTAM.*	CORRECTED† n_{th} ALONE	X-RAYS*	1 r OF X-RAYS EQUALS	1×10^{11} n_{th}/cm^2 EQUALS			OF n_{th} TO X-RAYS $(H_\gamma+N_p+B_\alpha+Cl_{\beta-\gamma})$	OF $p^+ + \alpha^{++}$ TO X-RAYS $(N_{p+}+B_{\alpha++})$
Isochromatid Deletions	0.1070×10^{-11}	0.07598×10^{-11}	0.003878	5.10×10^{9} n_{th}/cm^2	19.59 r	5.1×10^{8}	1.75	11.2/1	14.1/1
Chromatid+Isochromatid Deletions	0.2257×10^{-11}	0.1426×10^{-11}	0.01039	7.29×10^{9}	13.73 r	7.3×10^{8}	1.75	7.8/1	10.8/1
Chromatid Exchanges	0.04425×10^{-11}	—	$0.002416\ (R)^{1.5}$	—	—	—	—	—	—
Chromatid 50% Normal Cells Dose	2.05×10^{-11}	—	$(72_r-16.4\gamma$ contam.) $=55.6$ r	3.69×10^{9}	27.12 r	3.7×10^{8}	1.75	15.5/1	19.9/1
Chromosome 50% Normal Cells Dose	14.5×10^{-11}	—	(290 r − 116γ contam.) = 174 r	8.33×10^{9}	12.00 r	8.3×10^{8}	1.75	6.9/1	8.8/1
Chromosome Exchanges	0.02069×10^{-11}	—	$0.0149\ (R)^{1.75}$	—	—	—	—	—	—
Best Value	—	—	—	$\sim5\times10^{9}$ n_{th}/cm^2	$\sim$20 r	$\sim5\times10^{6}$	—	$\sim$11/1	$\sim$15/1

* See graphs, figs. 3, 4, 5.

† See text for correction for γ contamination.

‡ $\text{Equivalence} = \dfrac{C_{n_{th}}\ \text{alone}}{C\ \text{X-rays}}$.

§ See table 8.

‖ $\text{Efficiency} = \dfrac{\text{observed X-ray equiv. effect}}{\text{calculated rep.}}$

"Tolerance Flux" $= \dfrac{5\times10^{8}}{8\times60\times60} = 17{,}360\ n_{th}/cm^2$ per sec.

Such a correction for gamma rays is possible because it has been shown that unit doses of X- and gamma rays produce almost the same amount of these aberrations (KOTVAL, in LEA 1947, p. 239).

The equivalence of thermal neutrons to X-rays has been obtained by comparing the corrected thermal neutron effect with the X-ray effect for the same aberrations. The results of these comparisons are given in table 9.

If we assume the standard value for daily tolerance dosage, 0.1 r of X-rays per eight-hour day, to be that amount of any radiation producing an effect equal to this dose of X-rays, then the "tolerance dose" of thermal neutrons based on this biological effect is as shown in table 9.

We can in addition obtain an efficiency of thermal neutrons and X-rays in producing this biological effect by comparing the X-ray equivalent effect with the calculated amount of rep generated by a unit dose of thermal neutrons. This comparison has been made in the last two columns of table 9.

The observed biological efficiencies could be explained solely by errors involved in the estimate of the ionization dose absorbed in the tissue. This possibility has been considered but it is believed that the observed efficiency ($\sim$11/1) is too great to be accounted for by such errors, for the following reasons: the gamma ray contamination would have to be two and one-half times as great as estimated, the rep generated by thermal neutron capture six and one-half times as great, and the rep due to radioactive decay 80 times as great. Errors in the estimate of dose undoubtedly exist, but it is very unlikely that the estimated dose is wrong by two and one-half to six and one-half times, and therefore the efficiency observed is to be explained by other reasons, which will now be considered.

The comparison made in the last two columns of table 9 assumes that the inherent gamma-ray contamination has the same effect per unit dose as X-rays; further, the same is probably true for the hydrogen gamma rays and the chlorine^{38} beta and gamma rays, since they occur at random in the cells. This leaves us with the nitrogen protons and the boron alpha particles to consider, and apparently the combined $p^{+}+\alpha^{++}$ radiation has an efficiency to X-rays per unit of ionization of about 15/1 (last column, table 9). There are two lines of reasoning for believing that the protons plus alpha rays must indeed have this high efficiency in producing chromosomal aberrations. The first is the comparison of the amount of X-ray equivalent effect produced by a given dose of thermal neutrons with the calculated number of rep produced by the same dose. The second is based on the well established fact that the yield of the exchange or "two-hit" aberrations varies as a power (greater than 1) of the dose for X- and gamma rays, but linearly with dose for protons and alpha rays. If, indeed, the major part of the effect in this experiment is due to gamma radiation, which comprises 85 percent of all the ionization received by the tissue (table 8), we would expect that this preponderance of gamma radiation would be reflected in the exchange or "two-hit" aberration curve increasing more rapidly than the first power of the dose. On the contrary, it can be seen in the two thermal neutron graphs (figs. 3 and 4) that the frequencies of the chromosome and chromatid exchanges increase linearly with dose, but as the 1.5 power of the dose for the comparable X-ray

exposure (figure 5). Apparently the major part of the effect is produced by a radiation of heavy particles, as is concluded from the physical calculations.

The combined efficiency of the nitrogen protons and boron alpha rays must be about 15 times as great as X-radiation for the neutron dose to produce an effect equal to the observed X-ray equivalent dose. KOTVAL and GRAY (1948) have determined the efficiency of protons (from fast neutron recoils) and radon alpha particles to hard X-rays in producing the same aberrations in Tradescantia as are analyzed here. A comparison of the efficiency from external sources with their efficiency when originating internally from neutron capture shows:

RADIATION	EFFICIENCY TO X RAYS IN PRODUCING:	
	ISOCHROMATID DELETIONS	CHROMATID PLUS ISOCD.
1) From external sources:		
protons	3.7/1	2.6/1
alpha particles	7.8/1	4.1/1
expected from a beam of 62% p+38% α	5.2/1	3.2/1
2) Observed from thermal neutron beam of 62% p+38% α	14/1	11/1
3) Ratio: obs/expect.	2.7/1	3.4/1

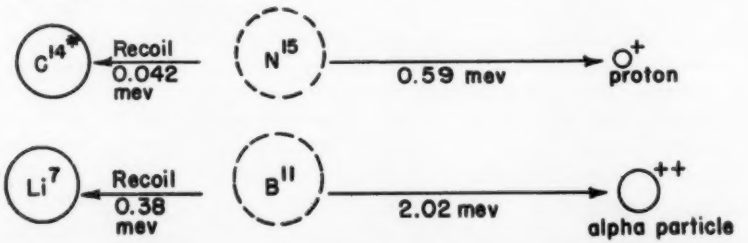
and it is clear that alpha particles and protons are more efficient when originating internally than when they come from external sources.[7]

This large and unexpected biological efficiency of protons and alpha rays resulting from thermal neutron capture, when compared with the efficiency of protons and alpha rays from external sources, suggests that there may be something which has been previously unconsidered or some difference between a biological reaction when the radiation enters the cell from without, and when it originates within the cell. Several mechanisms suggest themselves to account for this difference. It may be that the internal radiations cause chromosome breakage by indirect, in addition to direct, methods; this seems unlikely, however, since once emitted the particles behave the same as those coming from outside the cell. There is, however, one apparently important difference between particles coming from without the cell and those originating internally from thermal neutron capture. Particles coming from external sources

[7] The energies of the protons being compared are roughly about the same, 0.6 mev from nitrogen captures, and a spectrum of proton energies from the Li-D neutrons with 50 percent of the protons in the 0–1 mev range, 19 percent 1–2 mev 12 percent 2–3 mev, 4 percent 3–4 mev, etc. The α energies are quite different, 2.40 mev from boron captures, 5.49 from radon. The ion densities in tissue are about 2/1 ($\sim$2,500 ions/μ for boron α's, $\sim$1,200 ions/μ for radon α's), but in any case both of these α's have ion densities far in excess of the value generally accepted, $\sim$200 ions/μ, as the density necessary for the probability of chromosomal break to approach unity. The observed p^+ and α^{++} efficiencies are therefore probably not to be explained by these energy differences.

are distributed at random in the cell, but the particles originating from neutron capture are distributed as are the nitrogen and boron. Now, the frequency of chromosome breaks is dependent on the ratio of the target volume (the chromosomes) to the number of particles traversing the area; and if the concentration of nitrogen and boron per unit volume is greater in the nucleus and the chromosomes than the rest of the cell then the particles are not distributed at random and the number of "hits" on the chromosomes would be greater per unit dose than from randomly distributed protons and alpha particles. No definite data are available regarding the intracellular distribution of nitrogen, but apparently it is not unusually concentrated in the nucleus as a whole in rat liver cells (SCHNEIDER 1948) though it may be concentrated somewhat in the chromosomes. It seems likely that the boron may in fact be considerably more concentrated in the nucleus and chromosomes than in the cytoplasm, as observations of microincinerated resting stage cells have shown the bulk of the inorganic salts is concentrated in the nucleus with only small amounts thinly and evenly distributed in the cytoplasm (HORNING 1941). The constituents of these nuclear ashes have not been completely analyzed, but it seems reasonable that boron may be among them and is therefore more concentrated in the nucleus. Since the range of these 2.4 mev boron alpha particles is only 13 microns in tissue, and the diameter of the nucleus at the time these cells are exposed to radiation is about 12 microns,[8] there would be more particulate ionization in the nucleus than the rest of the cell. In addition, if a nitrogen or boron capture reaction occurs in a chromosome, the probability that either the particle or its recoil atom will traverse the chromosome and cause a break can be expected to be unity.

There exist two other likely explanations for the greater efficiency of internal to external originating protons and alpha particles, which are the recoil of the emitting atoms, and the transmutation of the emitting atoms. Let us consider the effect of the recoil atoms from the nitrogen and boron reactions. For both of these, the total energy of the reaction is divided between the particle and the emitting atom, so that the atom itself has energy and will recoil as is diagrammed below:



[8] Measurements have been made of the dimensions of the microspores used for these experiments. Cells have been selected which were at about the stage of development at which cells would be at the time of radiation—i.e., about four days before metaphase. About 100 cells were measured, from five different buds. Dimensions are as below (in microns):

Cells, outside dimensions: 32.1 long, 21.2 wide. Nucleus: 11.6 diam. Chromosomes (six, haploid): diam. 0.1, length 490. Chromosome dimensions are from SAX and SAX (1935).

Little is known about the range of these heavy recoil atoms, but certainly the energy is sufficient to break chemical bonds, and very likely is enough to break chromosomes. There is a likelihood that the dense ionization of the recoil atoms may increase chromosome aberration yield by injuring broken ends excessively so that the usual X-ray or neutron probability of chromosomal restitution is decreased; this seems to be true for alpha particles applied externally (KOTVAL and GRAY 1947).

Returning to the nitrogen and boron reactions given above, we see that whenever a capture reaction occurs, not only is a particle emitted and the atom recoiled out of position, but a new element is formed *in situ*, carbon from nitrogen and lithium from boron. This may be disastrous, particularly in the case of nitrogen which is an integral part of the protein molecules of the chromosome. The transformation of a nitrogen atom in a protein molecule into carbon may completely disrupt the molecule and possibly neighboring molecules. Almost nothing is known about the incorporation of boron in the cell, but it seems less likely that it is an integral part of the molecules of the chromosome.

Of the explanations given above for the differences of efficiency between external and internal particulate radiation, probably the most valid ones are the increased number of chromosome "hits" per unit dose because in the case of boron the emitting atoms are more concentrated in the nucleus and the chromosomes than in the cytoplasm; the recoil of the emitting atoms; the increase in the probability of a break by a traverse to unity; and the decrease of restitution probability by excessive injuring of broken ends by recoil atoms or alpha rays. The mere act of transmutation of an element in a chromosome seems to be an unlikely cause of chromosome breakage, though it may well account for gene mutation.

The daily "tolerance dose" and "tolerance flux" given in table 9 is understood to apply only to the production of this specific effect, chromosome aberrations, in a specific organism, Tradescantia. The temptation exists to apply the tolerance dose so derived to mammals and particularly to man but the physical and biological differences of the two cases make caution necessary in such a comparison. However, this study does demonstrate that there are several important factors which have not been considered in previous calculations of the tolerance dose and flux of thermal neutrons.[9] The first is the considerable contribution to the dose made by boron, which in this case is 29 percent of the total ionization dose due to capture alone. The second is that particulate radiation originating internally by neutron capture has an unusually high and unexpected efficiency in causing this biological damage, and is about three to three and a half times as damaging as the same dose of particulate radiation applied from without the cell.

[9] The apparent agreement between the tolerance flux of thermal neutrons derived from these experiments on Tradescantia and from the theoretical calculations of a tolerance flux for man derived by MITCHELL (1947) (tolerance flux Mitchell $1.24\times10^{4} n_{th}/cm^{2}$ sec; this experiment $=1.74\times10^{4}$) is purely fortuitous. The theoretical considerations of physical characteristics made by MITCHELL differ from the situation in the present experiment by numerous features.

SUMMARY

The cytogenetic effect of thermal neutrons from a special facility in the thermal column of the Oak Ridge nuclear reactor has been investigated. The production of chromosomal aberrations, both chromatid and chromosome types, in Tradescantia microspores has been compared with the production of the same aberrations by X-rays. Calculations of the number of roentgen equivalent physical absorbed from thermal neutron capture in tissue indicate that three elements, hydrogen, nitrogen and boron are responsible for about 99 percent of the ionization dose produced by neutron capture, and that about 77 percent of this dose is due to the protons and alpha rays from nitrogen and boron capture reactions. These conclusions are substantiated biologically by the observed linear increase of exchange aberrations with dose, which is characteristic of particulate radiation (with electromagnetic radiation, exchange aberrations increase as a power of the dose).

An equivalence of thermal neutrons/X-rays has been derived by comparing the production of chromosomal aberrations by the two radiations. The best value seems to be $\sim 5 \times 10^9$ thermal neutrons per $cm^2 = 1$ r of X-rays in biological effect; this would make the eight-hour tolerance dose (the same amount of effect as 0.1 r of X-rays) as based on this effect $\sim 5 \times 10^8$ thermal neutrons per cm^2.

A comparison of the X-ray equivalent effect produced by unit dose of thermal neutrons with the calculated number of roentgen equivalent physical generated by the same dose shows that thermal neutrons are about eleven times as efficient as X-rays per unit dose in producing this biological effect. If only the nitrogen protons and boron alpha rays from thermal neutron capture are considered, their efficiency compared with X-rays seems to be about 15/1. These biological efficiencies are about three to four times as great as have been observed when the same types of radiations were delivered from external sources. It is believed that this difference in the effectiveness of protons and alpha rays when they are administered to a tissue from an external source and these same radiations when they originate from the atoms of the tissue itself is not due to any difference in the effect of the protons and alpha rays themselves, but is due to a difference in their place and manner of origin.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the people of this laboratory who have assisted in the development of this study. Dr. Charles W. Sheppard has assisted in many of the calculations and given many valuable suggestions and criticisms throughout. Dr. William A. Arnold has helped in like manner, and derived the expression for the geometry of gamma radiation used in the calculation of rep. Dr. Waldo E. Cohn helped in the preliminary considerations of the components of dose. George Stapleton originated and accomplished the measurement of gamma contamination by the attenuation of gamma rays in lead. Cyrus Feldman of the spectroscopic section kindly made the spectrographic analysis of the anther material for the minor elements. Dr. Al Wattenberg of Argonne National Laboratory

furnished the activated gold foils used for the third determination of absolute neutron flux.

LITERATURE CITED

CATCHESIDE, D. G., D. E. LEA and J. M. THODAY, 1946 Types of chromosome structural change induced by the irradiation of Tradescantia microspores. J. Genet. **47:** 113–136.

GILES, N. H., JR. and H. P. RILEY, 1949. Proc. nat. Acad. Sci. **35:** 640–646.

HORNING, E. S., 1941 Microincineration and the inorganic constituents of cells. In G. Bourne, Cytology and Cell Physiology, Oxford: At the Univ. Press.

JONES, J. W., and R. T. OVERMAN, 1948 The use and calibration of a 100 percent geometry ion chamber. Atomic Energy Commission Document No. 2367.

KOTVAL, J. P., and L. H. GRAY, 1947 Structural changes produced in microspores of Tradescantia by alpha radiation. J. Genet. **48:** 135–154.

LEA, D. E., 1947 Actions of radiations on living cells. xii + 402 pp. New York: The Macmillan Co.

MARINELLI, L. D. 1942 Chromosomal effects of low X-ray doses on five-day Tradescantia microspores. Amer. J. Bot. **29:** 866–874.

MITCHELL, J. S., 1947 Provisional calculation of the tolerance flux of thermal neutrons. Brit. J. Radiol. **20:** 79–82.

SAX, H. J., and K. SAX, 1935 Chromosome structure and behavior in mitosis and meiosis. J. Arnold Arboretum **16:** 423.

SAX, K., 1940 An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics **25:** 41–68.

1950 The effect of X-rays on chromosome structure. J. Cell. and Comp. Physiol. **35:** Suppl. June.

SAX, K., and C. P. SWANSON, 1941 Differential sensitivity of cells to X-rays. Amer. J. Bot. **28:** 52–59.

SCHNEIDER, W. C., 1948 Intracellular distribution of enzymes. II. The oxidation of octanoic acid by rat liver fractions. J. Biol. Chem. **176:** 259–266.

WAY, K. and G. HAINES, 1948 Thermal neutron cross sections for elements and isotopes. Atomic Energy Commission. Document No. 2138.

THE GENETIC BASIS OF HETEROSIS IN MAIZE

F. G. BRIEGER[1]

Department of Genetics, University of São Paulo, Piracicaba, Brazil

Received December 27, 1949

THE literature on heterosis and the reduction of vigor after inbreeding has been reviewed recently from all angles by several authors (SPRAGUE 1946, RICHEY 1946, WHALEY 1944) and thus it is not necessary to enter into a detailed discussion, especially since I shall deal exclusively with the genetical aspect of the problem.

Up to now the phenomenon of heterosis in corn has been explained mainly by one of two alternatives: the hypothesis of dominance and that of heterozygosis. But in spite of continued discussion extending over a period of almost forty years, no conclusive evidence has been presented in favor of one or the other of these mendelian hypotheses.

The dominance hypothesis, first proposed apparently by BRUCE (1910)[2] and by KEEBLE and PELLEW (1910), postulates that the increase of vigor after crossing results from the combination of different dominant alleles, contributed by each parent. On the other hand, inbreeding again produces homozygosis for recessive alleles, causing a loss of vigor.

The heterozygosis theory, first formulated by SHULL (1911a, b,) and EAST and HAYES (1912) in rather vague physiological language, has been put in terms of modern genetic terminology by EAST (1936). According to this concept different alleles, when combined in heterozygotes, exert a complementary physiological action, resulting in an increased vigor limited to such heterozygotes. HULL's (1945, 1946, 1948) concept of overdominance seems, from the theoretical point of view, to be essentially the same as that of heterotic gene interaction.

As additional hypotheses we might mention other suggestions. BRIEGER (1930) pointed out that the nature of the inter-allelic interaction may be of the nature of dominant complementary factors. RASMUSSON (1933) made detailed references to the nature of quantitative gene interaction in producing heterosis.

[1] The present paper was written at the Division of Genetics, UNIVERSITY OF CALIFORNIA, Berkeley, under a special fellowship granted by the ROCKEFELLER FOUNDATION.

[2] There has been evidently some doubt about the priority in proposing the dominance hypothesis, and RICHEY (1945), reproducing BRUCE's original paper, points out that undoubtedly priority should be given to him. However, when reading carefully BRUCE's paper, it becomes quite evident that he missed the essential point of the dominance hypothesis, namely the covering up, in hybrids, of all or most recessive genes by their respective dominant alleles, contributed in part by one or by the other parent. The formulae given refer to a hybrid population obtained by crossing two parent populations which contain identical genes in different proportions, and it is shown that the mean frequency of heterozygotes in the hybrid population is inferior to the combined mean of the two parent populations. It would however be required that the hybrid population contains less homozygous recessives than either parent population individually, if we want to explain heterosis.

There cannot be any doubt that both of the two main hypotheses furnish satisfactory explanations of a large number of the facts. In order to overcome certain difficulties, JONES (1917) formulated his hypothesis of linked dominant factors, assuming that on the whole, dominant stimulating genes should be closely linked with recessive vigor-reducing factors. As a practical matter, this hypothesis occupies a somewhat intermediate position between the dominance and heterozygosis theories. If linkage between dominant and recessive genes became so close that practically no crossing over occurred, it would be difficult to distinguish experimentally between the linkage and the heterozygosis theory. We would be dealing simply with heterozygosis of chromosome regions, instead of that of simple genes. If on the other hand, linkage were rather weak, the difference between the simple dominance and the linkage hypothesis would practically disappear.

The main arguments mentioned in the literature against the dominance hypothesis are the following two:

a) A segregation of a simple system of dominant vigor producing factors should theoretically result in an asymmetrical segregation in the first inbred generation and this is not in accordance with the facts observed. COLLINS (1921) already pointed out that this asymmetry becomes practically obliterated if the number of segregating factor pairs is large and we may also refer to some curves given by BRIEGER (1930) which were calculated under the assumption that non-allelic interaction is simply cumulative, omitting the effect of phenotypic variability. With some 20 factor pairs in segregation, it would be practically impossible to verify statistically the asymmetrical nature of the distribution.

b) The second argument seems to me more serious. It is generally assumed under the dominance hypothesis that individuals exist, completely homozygous dominant for all loci, but that they are so rare that they cannot be discovered. If we start from an individual heterozygote for N loci, it is evident that in the nth selfed generation, without selection, there should be only $(\frac{1}{2})^n$ homozygous dominant descendants. If we attribute sufficiently high values to n, we can keep the value of this frequency so low that the absence of the homozygous dominants may appear fully explained. However, we have actually started from a wrong assumption. We should start from a completely homozygous dominant individual and ask, whether it is possible that a number of recessive alleles may be accumulated during phylogenetic development, sufficient to explain the appearance of highly heterozygous individuals and the complete elimination of the original homozygous dominant type. As I shall explain later in this paper, it seems impossible to obtain this result.

The main reason for the reluctance to accept the heterozygosis hypothesis seems to have been the scarcity of recognized cases of such a type of interaction, while today a considerable number of cases have been described. We may cite in the first place several examples of plants where the flower color in monofactorial heterozygotes is more intense than in either parent: *Silene Armeria*, pink × white gives red (CORRENS 1920), *Phlox Drummondi*, any cross between pink, rose or salmon gives scarlet, any shade of red crossed to blue gives purple, etc. (KOBAL unpublished). The F_2 segregation gives in these cases a simple 1:2:1 ratio. WRIGHT (1947) described a case in the guinea pig where a monofactorial heterozygote had a deeper brown coat color than either homozygote. DUNN and CASPARI (1945) found that heterozygotes for the alleles l^0 and l^1 are normal, while l^0l^0 and l^1l^1 homozygotes are lethal genotypes. KERR (1948) explained the fertility of the queens in Melipona as being due to their heterozygosis for two or for three pairs of factors, the number of fac-

tors varying in different species. STERN and his collaborators (1943, 1946, 1948a, b) described cases in *Drosophila melanogaster*, where heterozygotes in the series of position alleles at the cubitus interuptus locus show a higher degree of abnormality in wing venation than either homozygotes or hemizygotes.

Several cases are known of monofactorial and even of bifactorial segregations where the heterozygotes have a higher survival value than either homozygotes: barley (GUSTAFSON 1946, 1947), Antirrhinum (STUBBE and PIRSCHLE 1940), and we may include also here the cases reported by SINGLETON (1943a, b) and JONES (1944, 1945) though the latter cases have not been carefully analyzed.

Finally, a number of cases are now known in Drosophila where monofactorial heterozygotes (L'HERITIER and TEISSIER 1933, 1934, 1937a, b; TEISSIER 1942, 1944, 1947a, b; KALMUS 1945) or heterozygous inversions (Dobzhansky 1943 to 1947, WRIGHT and DOBZHANSKY 1946) have a higher selective value than either homozygotes.

It is only very recently that the question of heterosis has been discussed from the point of view of population genetics. Simultaneously CROW and BRIEGER presented papers to the Genetics Society of America and the Societade Brasileira Biologica in 1947 (CROW 1948, BRIEGER 1948a and b). As we shall see later, both the discussions lend support to the heterozygosis hypothesis.

The behavior of individual maize plants on crossing or selfing is in general agreement with either the dominance or heterozygosis hypothesis. But results that I obtained when studying indigenous South American maize which have been cultivated for a very long time without the aid of modern genetical methods, led me to study the evolutionary origin of heterotic gene systems, under the effects of natural or artificial selection. The result of this study was a new approach to the heterosis problem, which in turn led to a new breeding procedure that has already been used in the production of the new Brazilian sweet corn (BRIEGER 1948b).

The evolutionary or phylogenetic aspect of heterosis seems to have been largely overlooked. COLLINS (1918) postulating that corn was derived from a hybrid between Euchlaena and some wild Andropogoneae, assumed that the latter already possessed the heterotic gene system. In considering the extreme reduction in vigor following selfing of maize BRIEGER (1944) suggested that it might be explained by recessive lethals having become established as balanced lethals in an original interspecific hybrid, owing to a mutual incompatability of these genes of either species when homozygous.

The main object of the present paper is twofold. First, to determine the method by which a genic system responsible for heterosis could arise by normal evolutionary processes, and secondly, to decide whether or not the reduction of vigor following inbreeding is in accord with known principles of population genetics.

DEFINITION OF THE TERM HETEROSIS

Before entering into a more detailed discussion it is necessary to define "heterosis." When SHULL introduced it in a lecture given in 1914, but pub-

lished for the first time in 1922, his intention was to follow JOHANNSEN'S method when the latter defined the gene, that is: to propose a term for the phenomenon of hybrid vigor free from any previous theoretical concept (1948).

To begin with, we may state that heterosis is generally considered to be present when the mean of any character or characters in a hybrid exceeds the mean of its descendants obtained by any system of close inbreeding.

From a purely formal point of view, we may expect that the transgression of the mean of heterozygotes, beyond the range of the means of homozygotes, may be in either direction, that is, in what we may call a positive or negative direction. But at least for the time being it seems to me unnecessary to distinguish between positive and negative heterosis. From a biological point of view, it is immaterial where we locate the zero point and which direction we choose to call positive or negative. The cases reported by STERN and his collaborators (1943, 1946, 1948a, b) may be considered examples of negative heterosis since heterozygotes are less normal or, if we prefer to say, more abnormal than the homozygotes. In selecting for height of plants in maize, we may take our measurements of height with the zero point at the basis of the plant or with the zero point at a value which corresponds to the mean height of the plant. If we want to obtain smaller plants, as is frequently the case in South American varieties, we may indicate deviations in the undesirable direction, that is, an actual increase of height, by a negative sign, while decrease in height in the desired direction would be considered as a positive result and thus characterized by a positive sign.

In order to avoid the impression that there are two fundamentally different kinds of heterosis, a positive and a negative one, one should select the scale of measurements in such a way that the transgression of the mean of heterozygotes appears positive. However, a blind application of this principle may lead to absurd conclusions, for instance, the increased vigor of many interspecific hybrids may be justly considered as positive heterosis, but a reduction of vigor or fertility which also occurs in interspecific crosses quite frequently is an entirely different problem and cannot be considered as "negative heterosis."

There are some additional considerations which we have to make:

A) Heterosis must refer to the comparison between characters of hybrids and their descendants, obtained through inbreeding.

Since the phenotype of hybrids depends largely upon the complicated interaction between alleles at the same or at different loci, the presence of heterotic gene action in hybrids may frequently become quite obscure. If we cross two inbred lines of different varieties, the effect of heterosis may become much more pronounced than the result of gene interaction in general, but if instead, we use two hybrids between inbred lines of either variety for obtaining the varietal hybrid, then frequently, the latter will be no more vigorous than its parents, which were themselves hybrids. In other words, the mean values for quantitative characters of an intervarietal hybrid may not be superior to the means of the corresponding intravarietal hybrids, but they will show the presence of heterosis when compared with the means of inbred

lines. It is thus evident that the comparison between hybrids and their parents will not always yield a reliable criterion on which to base a definition of heterosis.

If, however, we compare hybrids with their offspring a reduction in vigor will always be observed whenever the hybrids were heterotic. This reduction in vigor is generally quite pronounced in the first inbred generation of intravarietal crosses. In intervarietal crosses, ordinary mendelian segregation for quantitative differences may obscure the reduction in vigor in the first or the first two generations, but it will always appear in subsequent generations.

Thus we may formulate the following definition: Heterosis is characterized by an increase of the mean value for quantitative character differences, when comparing hybrid means with those of their offspring obtained by selfing or any other method resulting in close inbreeding. Heterosis may also cause an increase of means of hybrids over their parents. The main and most important feature of heterosis is the impossibility of maintaining hybrid vigor in the offspring obtained by any kind of inbreeding.

B) Heterosis does not affect the individuals as a whole, but the expression of each character, as a rule, independently of that of other characters. Thus it is not correct to expect a plant on the whole to be heterotic.

Heterosis in maize affects mainly the following characters: plant height, position of the ear, size of leaves, intensity of chlorophyll formation, size and strength of root system, resistance to disease, pests and unfavorable conditions, size and number of kernels, width and length of ear, size and branching of tassel, and amount of pollen shed. Inbred plants may grow somewhat slower than the corresponding hybrids, but earliness and lateness is an ordinary quantitative and not heterotic character, with earliness being completely or partially dominant. Row number in the ear is not affected by heterosis, heterozygotes having in general an intermediate row number. Plant and kernel color show a typical mendelian qualitative or quantitative behavior. The degree of heterosis may be quite different in individual cases. For instance, South American indigenous corn shows generally a very pronounced reduction in ear size of the first inbred generation, while the plant height may not be affected to the same degree.

Recently there has been a tendency to use the terms heterosis and high adaptive value as identical. The relative adaptive value of any character depends evidently upon the type of selection applied. Thus a new criterion is introduced which in itself is independent of the nature of the heterotic character itself. For instance, plant height is a typical heterotic character, using the ordinary definition. If maize has to compete in badly cultivated fields with weeds and the brush coming up from roots of burned forest trees, then an excessive height of the plants will represent a high adaptive value. But if we keep the corn fields clean, the excessive plant height, with ears far from the ground, may produce an extreme degree of lodging, and thus would be an undesirable character. Taking the degree of adaptation to natural or artificial conditions as a decisive criterion, the second case could no more be called heterotic. We may cite another example: the Indians in the Choco, a region

of Colombia, plant their maize broadcast, and under such conditions plants which are too vigorous would tend to be eliminated. But under ordinary conditions of a mixed maize-bean planting, vigorous stalks which can support the weight of the bean vines, are selected. Thus we see that an ordinary heterotic character such as plant height will sometimes have a positive and sometimes a negative adaptive value.

In conclusion, I may thus say that it seems to be best to avoid any subjective criteria and to consider heterosis to be present in those cases in which any quantitative character shows a higher mean in the hybrid generation than in any of the subsequent generations, obtained by any form of close inbreeding.

PRINCIPLES OF POPULATION GENETICS, SELECTION AND MUTATION

In a population which contains the three genotypes AA, Aa and aa, their relative frequency will depend mainly upon three factors: a) their constancy, that is, the rate of mutation, b) the reproductive system, whether random mating, preferential mating or selfing, and c) the relative survival values of the three genotypes. First we shall discuss the latter in some detail.

In order to obtain index values for fitness we must accept one of these three genotypes as a standard with a survival value as unity. We find in the literature numerous notations. Thus WRIGHT (1931) sometimes uses the letter s to denote the selective value of a recessive lethal or a partially lethal mutant, and $h \cdot s$ for the selective value of intermediate heterozygotes. In the case of heterosis he uses the letters s and t for the selective values of both homozygotes (1931, 1947). The standard value is represented in the first two cases by the viability and fertility of the homozygous dominant individuals and in the third case by that of the heterozygote. The corresponding survival values will then be $(1-s)$, $(1-h \cdot s)$, etc. HALDANE used a similar procedure (1926) for heterotic cases, using however different letters of the alphabet. I shall use here, as in some previous publications, still other letters, in order to avoid confusion since there are some apparent differences in the biological definition given by the authors mentioned. If we admit that survival indexes may take any positive value and may be larger or smaller than unity, then there is no need to use special letters for individual genetical cases. I proposed to use (1948a) uniformly the letter R to denote the individuals or gametes remaining free to function in a population after all selective processes have had played their part, accepting as standard the survival value of the monofactorial heterozygotes. The survival values of the homozygotes are calculated as indexes in reference to this standard or unit value. If the heterozygote should be less viable than one of the homozygotes, the survival values of the latter would be larger than one, and if there should be heterosis for survival values, the indexes for both homozygotes will be smaller than one.

The survival values, as just defined, may actually be determined in special experiments. Suppose we plant equal numbers of individuals of all three genotypes and are able to count the total number of gametes produced by all surviving individuals of each genotype, we may obtain numbers which we may indicate by the letters x, y and z. Dividing the triple proportion of genotypes

after selection by the value y, we obtain a mathematical definition of survival values.

Initial phase:

$$1\,(AA):1\,(Aa):1\,(aa).$$

After all selective processes:

$$x\,(AA):y(Aa):z(aa)$$

or finally

$$\left\{\frac{x}{y} = R_A(AA)\right\}:\left\{\frac{y}{y} = 1(Aa)\right\}:\left\{\frac{z}{y} = R_a(aa)\right\}.$$

If the survival indexes of the two homozygotes are different, we shall use the capital letter A as a subscript to denote the higher index and the letter a to denote the lower of the two indexes.

These total survival indexes R are compound values, if studied biologically in some detail. If we take the whole vegetative phase, only a certain fraction of individuals will reach reproductive age, and this fraction may be denoted by the symbols R_{AV} and R_{aV}. Furthermore only part of the gametes of the surviving plants may function and we shall denote the remainder, after reproduction selection by the letters R_{AR} and R_{aR}. Mathematically these two partial survival values are related by a very simple equation:

$$R_A = R_{AV} \cdot R_{AR};\ R = R_{aV} \cdot R_{aR} \cdots \tag{1}$$

The partial survival values may be still further subdivided, if necessary. We may, for instance, determine successively the frequency or the percentage of seeds with good embryos, of good seeds which germinate, of plants surviving seedling stage and of plants reaching flowering stage, etc. If we design these successive fractions using numbers as subscripts, we obtain the following subdivision of vegetative survival indexes:

$$R_{V1} \cdot R_{V2} \cdot R_{V3} \cdot R_{V4} \cdots R_{Vn} = R_V.$$

Furthermore, we may determine the causes of elimination at each of the above mentioned stages, for instance, how many seedlings are not albinos, but green, how many of the green seedlings are dwarfs, etc. Using letters as subscripts, it is easy to see that these partial survival values are additive:

$$R_{V2a} + R_{V2b} + \cdots = R_{V2}.$$

In order to give a better idea of the importance of the subdivision of the total survival indexes we shall give some special cases. For instance, it is quite common that inbred lines of maize show a reduction in the percentage of germinating kernels, and that ears and tassels and the number of ovules and of pollen grains is considerably reduced. A vegetative survival value of .75 caused by imperfect germination, and a reproductive survival index of .5 in consequence of the reduction in size of ear and tassel, could not be considered as very low, but when combined, they result in a rather low total survival

index: $.75 \times .5 = .375$. In plants such as Capsicum inbreeding results also in a loss of vigor and here we may readily obtain a percentage of germination of about .7, a slight reduction of the flower formation of the order of .7 and a further reduction in the number of seeds in the fruits formed of about .6, always compared with the same characters in hybrids. Thus, we obtain a low total survival value of $.7 \times .7 \times .6 = .294$.

In numerous discussions on the effect of selection, very low selective values and thus very high survival values have been used when calculating demonstrative curves, and the corresponding survival values were actually not very different from one. The two examples given above show that even when the individual partial survival values are quite high, the total survival value turns out to be rather low.

Next we shall briefly discuss *mutation rate*. Though there are few data at our disposal about the actual mutation rate in maize at different loci, a rate of the order of 10^{-4} to 10^{-6}, with a most probable average of 10^{-5}, seems to be acceptable as a preliminary estimate.

The number of mutable loci is another point to which we shall refer repeatedly. Though there may be a total of the order of 5,000 loci present in maize, it seems to me dubious if we can follow CROW (1948) and suppose that all of them may mutate to one type of allele, i.e., to recessive lethals or subviables. We may accept as a general rule that new mutant genes are recessive in their most conspicuous phenotypic effect. We cannot, however, expect that all mutations will simply reduce survival.

The different phenotypic effects which may cause a loss of vigor can be attributed to subviable mutant genes, such as deficient seed formation, deficient germination, deficiency in chlorophyll formation, etc. Thus we can subdivide the total of subviable mutants into groups. There are hardly more than a few hundred loci in maize responsible for any one of these different ontogenetic phases.

With regard to the *reproductive system* we only have to distinguish in the present publication between the most extreme possibilities. A population of maize plants may be considered as a random-mating breeding unit, where the pollen of any plant has equal chances to reach the silks of any individual in the population, including that of the same plant. Actually this assumption is not quite correct for two reasons: The chances for natural self-pollination are somewhat reduced. That is, there is less chance for self-pollination than for cross-pollination because there is frequently a slight difference in flowering time between tassel and ear of one individual and furthermore the pollen rarely falls in a vertical direction. Also the chances of cross-pollination are not strictly random and decrease with increasing distance between plants. But for the present discussion both these limitations may be disregarded. In a lot of several thousand plants, the chance of self-pollination is insignificantly low anyway, and the effects of the variation in distance between plants will be without importance if all different genotypes in a field of maize are distributed at random.

Self-pollination on the other hand must be carried out artificially in maize

and this selfing can be done in such a way that we avoid all experimental errors which may lead to contamination and to crossing which would result in an incomplete system of inbreeding.

POPULATION EQUILIBRIUM IN A RANDOM MATING POPULATION

The formula for determining the proportion of two alleles at equilibrium in a population can be derived by a simple process. The gametic frequencies at equilibrium in generation n must be the same as that in the following generation (n+1), in spite of the occurrence of new mutations and of all selective processes. Let us assume that the final frequency of two alleles A and a in generation n was equal to p and q respectively. Following the HARDY-WEINBERG law we should have in the next generation the following proportion of phenotypes:

$$p^2(AA) + 2pq(Aa) + q^2(aa).$$

After all selective processes have played their part again, and without the occurrence of any mutation this sum reduces to:

$$p^2 \cdot R_A(AA) + 2pq(Aa) + q^2 \cdot R_a(aa).$$

Furthermore if a mutation occurs from A to a at the rate u, and without any selection, the original HARDY-WEINBERG formula suffers the following alteration:

$$(p - pu)^2(AA) + 2(p - pu)(q + qu)(Aa) + (q + pu)^2(aa).$$

On the whole we are justified not to consider the occurrence of recurrent mutations from a to A, its frequency v being considerably smaller than u. If however we want to include this phenomenon in the formula, we must add the term qv to the gametic frequency of p and subtract it from that of q.

If we want to combine both processes, mutation and selection, we encounter certain difficulties. If a mutation occurs in a homozygote (AA), giving rise to a heterotic heterozygote (Aa), this would cause an alteration of survival value. The homozygote underwent only part of the total selection before undergoing mutation, and thus we should use in the formula for those homozygotes, which will mutate eventually, a partial survival value R_A' which is larger than R_A. On the other hand, if a mutation should occur in a heterozygote (Aa) resulting in the appearance of a homozygote (aa), this in turn would have again an effect on survival. The new homozygote will have again a partial survival value larger than the usual survival value R_a of homozygotes (aa). We may, however, assume that on the whole both these partial survival values will be so near to the value one, that we may disregard them.

Thus, combining the two formulas given above, and remembering that after meiosis the heterozygotes segregate into equal proportions of gametes (A) and (a), we may write the formula at equilibrium, with selection and mutation:

$$\frac{p}{q} = \frac{p^2R_A + pq - p \cdot u}{q^2R_a + pq + p \cdot u}. \qquad (2)$$

By simple algebraic transformation this proportion reduces to:

$$\frac{p}{q} = \frac{(1 - R_a) - u/q^2}{(1 - R_A)} \tag{3a}$$

or, if we prefer, we may write also:

$$u = (1 - R_a) \cdot q^2 - (1 - R_A) \cdot q \cdot (1 - q). \tag{3b}$$

We may use these formulas to decide whether the dominance hypothesis or the hypothesis of a heterotic gene interaction is correct. It is generally agreed that the heterotic mechanism in maize has been established long ago. Thus we must assume that such mutations, either to recessive genes, which reduce viability and which we may call subviables, or to heterotic genes, have been slowly accumulated and have reached a population equilibrium, governed by the formulas given above. It it should be shown that such a phylogenetic accumulation is impossible for certain assumed values of R, then the hypothesis used in estimating these values of R must be rejected.

We shall start with a discussion of the *dominance hypothesis*. Here the survival value of the homozygous dominant individuals (R_A) is equal to that of the heterozygotes (*Aa*), both being unity. The homozygous recessive subviable mutants will have a survival value R_a, smaller than 1. Under these conditions the second term in formulae (3b) becomes equal to zero and vanishes, and we come actually to HALDANE's formula for recessive subviable mutants:

$$u = (1 - R_a) \cdot q^2 \quad \text{or} \quad q = \sqrt{\frac{u}{(1 - R_a)}}. \tag{4}$$

The limiting extreme values are:

Recessive lethal mutants:	Recessive neutral mutants:
R_a = zero	R_a = unity
$q = \sqrt{u}$	q = infinite
$p = 1 - q = 1 - \sqrt{u}$	p = zero

The frequency of the different homozygous and heterozygous genotypes per locus in a population at equilibrium can be easily determined, and the mean frequency for any number of mutable loci can be obtained by multiplying the individual frequencies per locus by the number of loci involved. Table 1 gives the individual frequency of monofactorial recessives and heterozygotes, for a series of mutation rates for four different types of subviable mutant genes.

The next step must consist in establishing limiting conditions which enable us to decide whether the dominance hypothesis is in accord with the facts known. We can establish the following requirements: (a) no homozygous dominants should occur in the balanced random mating population; (b) the inevitable reduction of vigor after selfing requires that every individual of the

population should be heterozygous for at least one or more loci; (c) the absence of an asymmetrical distribution requires that every individual of the population is heterozygous for numerous loci.

All these requirements will be fulfilled if we can be sure that any individual obtained by selfing will contain at least one or more subviable mutant genes in the homozygous recessive condition. Thus we have to solve the question: which Poisson series does not reach, within reasonable probability limits, the value zero. The answer is a series with mean 7 where 99 percent of the

TABLE 1

Frequencies of recessive alleles and of heterozygotes at different mutation rates for four different types of subviable mutant genes of one locus.

MUTATION RATE	GAMETIC FREQUENCY OF REC. ALLELE	ZYGOTIC. FREQUENCY OF HETEROZYGOTE
	$R_a = .9$	
10^{-4}	.0316	.0612
10^{-5}	.0100	.0198
10^{-6}	.0032	.0063
	$R_a = .75$	
10^{-4}	.0200	.0392
10^{-5}	.0063	.0125
10^{-6}	.0020	.0040
	$R_a = .5$	
10^{-4}	.0141	.0280
10^{-5}	.0040	.0080
10^{-6}	.0014	.0028
	$R_a = 0$	
10^{-4}	.0100	.0188
10^{-5}	.0032	.0063
10^{-6}	.0010	.0020

total distribution lies between the limits 1 and 15. Thus we must require that any plant of the original population is heterozygous for at least 4 times 7 or 28 loci.

This requirement leads us to the next argument: We must now find the mean of another Poisson series which does not go below the value 28 within reasonable probability limits.

Next we must decide what we should accept as such a probability limit. I am using in the first part of the discussion the limits of 10^{-2} and 10^{-4}. Any genotype expected with frequencies higher than these limits is either very likely or at least reasonably probable to occur. Before stating final conclusions, however, these limits must be defined in a still more rigorous way. Everybody agrees that no homozygous dominant individual of maize has yet been found, and it is thus an essential question to estimate how rare such individuals must be in order to have escaped notice—if they should really exist.

Personally I have probably seen, in my experiments, offspring from only about 7,000 selfed individuals. If one allows similar or higher figures for every geneticist or breeder of the last 30 years, the total number of selfed offspring studied would correspond to more than a few hundred thousand selfed individuals. Thus, I think, we are justified in stating that any constitution expected with a frequency of more than once in a million had a good chance to come under observation. We should further add to the evidence studies on the indigenous corn, though it is hard to make a safe estimate as to numbers. I have studied during the last 13 years about 1,000 families from indigenous selfed material without finding any homozygous dominants.

The argument is thus divided into parts: first using the lower and preliminary limits and then passing to the high limit of probability.

I shall consider three types of mutants: Lethals, medium subviable mutants with R = .5 (s = .5), and weak subviable mutants with R equal to .9 (s equal .1) (table 2) and a mutation rate of 10^{-5}.

TABLE 2

TOTAL OF MUTABLE LOCI	NUMBER OF HETEROZYGOUS LOCI PER INDIVIDUAL AT EQUILIBRIUM		
	MEAN	EXTREMES AT 1/100 PROBABILITY LEVEL	EXTREMES AT 1/10,000 PROBABILITY LIMIT
	Lethal mutants: R=0		
1,000	6.3	1 — 13	0 — 15
3,000	18.6	8 — 28	4 — 36
5,000	31.5	18 — 45	12 — 55
	Subviable Mutants: R=.5		
1,000	9	2 — 17	0 — 23
3,000	27	14 — 40	9 — 49
5,000	45	25 — 68	21 — 73
	Subviable Mutants: R=.9		
1,000	20	8 — 28	4 — 35
2,000	40	25 — 56	18 — 67
2,500	50	33 — 68	25 — 79
3,000	59	41 — 79	32 — 96

From table 2 we can conclude that considerably more than 5,000 loci, mutable to subviable genes with survival index R equal or smaller than .5, are necessary if we want to reach a sufficient degree of heterozygosis of at least 28 heterozygous loci.

We might have reached the same conclusion in a shorter way, but it may have been interesting for those, not familiar with Poisson series, to have first individual limits. But we may have simply asked what are the means for Poisson distributions which do just reach, at different probability levels, the value 28, and calculate then the total number of loci which correspond to these Poisson means (table 3).

TABLE 3

PROBABILITY LEVEL	MEAN OF POISSON SERIES	CORRESPONDING TOTAL NUMBER OF MUTABLE LOCI		
		$R_a = .9$	$R_a = .8$	$R_a = .7$
10^{-6}	63	3.150	4.500	5.530
10^{-5}	57	2.850	4.070	5.000
10^{-4}	54	2.700	3.860	4.740
10^{-3}	49	2.450	3.500	4.300
10^{-2}	42	2.100	3.000	3.680

Thus we require from 3,000 to 5,000 loci changing, with a mutation ratio of 10^{-5}, to recessive, with survival index above .7, and more loci still if the survival index should be below .7, in order to fulfill our minimum requirement or with other words, we need almost all loci present.

I do not think we can dispose of all loci in this way. We know that a considerable number of loci yield mutants with much smaller survival values (defective seeds, albino and luteus seedlings, barren stalk, silkless, etc.). Furthermore we know that any maize plant contains a large number of modifier genes with no appreciable effect on survival values, as can be shown easily by selection experiments for shifting phenotypic expressions of any character. I stated above that heterosis is a complex phenomenon, forcing us to distinguish and to treat to some extent independently the heterosis in mean plant height, in mean ear size, in mean kernel weight, etc. There are evidently not enough mutable loci in maize, sufficient in number to build up the necessary genic system with only recessive subviable mutations.

Thus I think we have to reject the dominance hypothesis as an exclusive or general explanation of heterosis in maize. There seems to exist no possibility of accumulating a number of heterozygous factor pairs, sufficient for the phenomena observed and which we set out to explain.

On the other hand, the data seem to me in accordance with the estimated actual frequency of recessive subviable mutations which appear in South American maize after inbreeding, if we assume that there exist only a few hundred or less mutable loci for any phenotypic group of mutations. The frequency with which cytological aberrations appear in inbred lines is also of the same low order. These abnormalities cannot be the cause of heterosis or of the reduction of vigor after inbreeding as was supposed by MÜNTZING (1946), but rather the consequence of the breeding system.

CROW (1948) has reached a somewhat different conclusion. Since the frequency of homozygous subviable recessives is equal, at equilibrium, to u/s, one may say that the fraction lost, due to the presence of the homozygous subviable mutations, is equal to $s \cdot u/s$ or u. Assuming that there are N mutable loci, the total loss would be equal to $N \cdot u$, which gives for a mutation rate of 10^{-5} and 5,000 mutable loci a total average loss of .05. From this CROW concludes that the dominance hypothesis would be a sufficient explanation of heterosis, if ordinary random mating populations would yield, at the most,

TABLE 4

SURVIVAL INDEXES		ZYGOTIC RATIOS									GAMETIC FREQUENCY
		REPRODUCTIVE ELIMINATION			REPR. AND VEGT. ELIMINATION			VEGETATIVE ELIMINATION			
R_A	R_a	*AA*	*Aa*	*aa*	*AA*	*Aa*	*aa*	*AA*	*Aa*	*aa*	*A*
Frequencies at equilibrium in random mating populations											
.8	.8	44.44	50.00	25.00	23.68	52.64	23.68	22.22	56.56	22.22	50.00
	.6	44.44	44.44	11.11	42.86	47.62	9.52	41.03	51.26	7.69	66.67
	.4	58.25	37.50	5.25	55.10	40.82	4.08	52.94	44.12	2.94	75.00
	.2	64.00	32.00	4.00	63.16	35.09	1.75	60.95	38.10	.95	80.00
	.0	69.44	27.78	2.75	—	—	—	66.67	33.33	—	83.33
.6	.6	25.00	50.00	25.00	22.22	55.56	22.22	18.75	62.50	18.75	50.00
	.4	36.00	48.00	16.00	33.33	55.56	11.11	28.42	63.16	8.42	60.00
	.2	44.44	44.44	11.11	42.11	52.63	5.26	36.36	60.61	3.03	66.67
	.0	51.02	40.82	8.16	—	—	—	42.85	57.15	—	71.43
.4	.4	25.00	50.00	25.00	18.75	62.50	18.75	14.29	71.43	14.29	50.00
	.2	32.65	48.98	18.37	25.80	64.52	9.68	19.88	74.54	5.59	57.14
	.0	39.06	46.88	14.06	—	—	—	24.99	75.01	—	62.50
.2	.2	25.00	50.00	25.00	14.29	71.43	14.29	8.33	83.33	8.33	50.00
	.1	28.02	49.83	22.15	16.76	73.30	9.93	9.72	86.43	3.84	52.94
	.0	30.86	49.38	19.75	—	—	—	11.11	88.89	—	55.56
.1	.1	25.00	50.00	25.00	11.54	76.92	11.54	4.55	90.90	4.55	50.00
	.05	26.37	49.96	23.67	12.63	79.81	7.56	4.90	92.90	2.20	51.35
	.00	27.70	49.86	22.44	—	—	—	5.26	94.74	—	52.63
Frequencies at equilibrium in selfed populations											
.5	.5	50.00	—	50.00	50.00	—	50.00	50.00	—	50.00	50.00
	.4	100.00	—	—	100.00	—	—	100.00	—	—	100.00
.4	.4	41.67	16.67	41.67	37.50	25.00	37.50	33.33	33.33	33.33	50.00
	.2	57.69	23.08	19.23	52.94	35.30	11.76	46.15	46.15	7.69	69.21
	.2	62.50	25.00	12.50	—	—	—	50.00	50.00	—	75.00
.3	.3	35.71	28.57	35.71	27.78	44.45	27.78	21.43	57.14	21.43	50.00
	.2	40.54	32.43	27.03	31.92	51.06	17.02	24.32	64.86	10.82	56.76
	.0	45.45	36.36	18.18	—	—	—	27.27	72.73	—	63.63
.2	.2	31.25	37.50	31.25	20.00	60.00	20.00	12.50	75.00	12.50	50.00
	.0	35.76	42.83	21.43	—	—	—	14.31	85.69	—	57.17
.1	.1	27.78	44.44	27.78	13.64	72.72	13.64	5.56	88.89	5.56	50.00
	.0	29.41	47.06	23.53	—	—	—	5.88	94.12	—	52.94

only five percent less than the maximum possible in a hybrid population not containing any homozygous recessive individuals. I think one may safely say that most populations of open pollinated field corn yield considerably less than 95 percent of maximum yield possible. If it were not so, it seems most unlikely that breeders would have resorted to the complicated and rather expensive process of producing hybrid corn which certanly would not be an economic proposition unless the gain was considerably more than five percent.

Next we shall turn back to formula (3a) and study the situation for *heterotic mutants*, i.e., mutation to genes which cause the heterozygotes to have a higher survival value than either the original or normal genes or the new subviable mutants. Since the term (u/q^2) can now be considered as generally very small when compared with the usual values of R_A and R_a, the formula may be simplified and written as given by several authors:

$$\frac{p}{q} = \frac{1 - R_a}{1 - R_A} . \tag{5}$$

If we want to calculate the zygotic frequencies, we cannot apply the HARDY-WEINBERG rule in its simple form. We have to take into consideration that generally individuals are observed after they have undergone at least part of the vegetative selection. Thus we must write (BRIEGER 1948a) the triple proportion:

$$\begin{array}{c} AA \\ \hline Aa \\ \hline aa \end{array} \quad \begin{array}{c} (1 - R_a)^2 \cdot R_{AV} \\ \hline 2(1 - R_a)(1 - R_A) \\ \hline (1 - R_A)^2 \cdot R_{aV} \end{array} . \tag{6}$$

Formulas (5) and (6) show that population equilibrium will be reached independently of the original gene frequencies or the mutation rate. Table 4, upper part, repeated after BRIEGER (1948a) contains the gametic and zygotic proportions obtained at equilibrium. It is evident from this table that for survival values of homozygotes smaller than .6, we have about 50 per cent or more of monofactorial heterozygotes in the population at equilibrium. If a plant should be heterozygous for one locus only, one half of its descendents after selfing will maintain the vigor and the other half will be less vigorous than the parent. If we should require that practically no descendent should have the same vigor as the parent individual, it would be sufficient to assume the presence of ten heterotic loci.

It should also be noted that equilibrium will generally be reached rather rapidly. From tables and figures of the publication already mentioned (BRIEGER 1948) we can take the following example.

If mutations to a heterotic mutant gene occur at a rate of 10^{-4} with survival values of $R_A = .6$; $R_a = .4$, it only takes about 9 generations for this mutant to reach a frequency of 10^{-2} or one percent. In another 10 generations equilibrium will be reached where the population contains 60 per cent of original genes and 40 percent of the mutant gene. If the mutation rate was only

10^{-6}, it will require about 18 generations to reach a one percent level for the new mutant gene and another 10 generations to reach equilibrium.

The superiority of a system of heterotic mutants as compared to a system of dominant recessive genes can be shown more directly in the following way:

Figure 1 illustrates the frequencies of heterozygotes at equilibrium, (a) in a population in which 20 loci have mutated to heterotic allelic pairs, with average

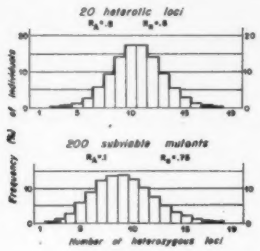


FIGURE 1

survival values of $R_A = .80$, $R_a = .60$ and (b) in a population in which 200 loci have mutated at a rate of 10^{-4} to subviable recessives, with an average of $R_A = .1$, $R_a = .75$. The two distributions are approximately equal, the mean number of heterozygous loci being 9.4 in the case of heterotic loci and 8.0 in the case of recessive subviable loci. We may conclude that it would require about 200 loci

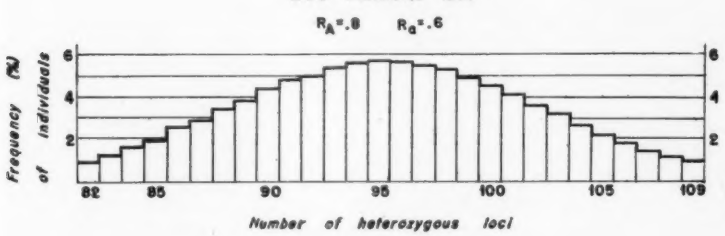


FIGURE 2

which are capable of mutating to recessive subviable alleles, to give about the same degree of efficiency as 20 loci which had mutated to heterotic subviable alleles. Figure 2 shows the frequency distribution, calculated for 200 loci, containing subviable heterotic mutations. On the average, an individual contains 95 heterozygous and 105 homozygous loci.

Finally the following summary shows in a very decisive manner the greater efficiency of heterotic gene pairs over subviable or lethal recessive mutants. (The numbers given refer to the total of mutable loci necessary in order to obtain, at equilibrium, 50 or 100 heterozygous loci.)

TYPE OF MUTANT	NUMBER OF MUTABLE LOCI	
	50 HETEROZYGOUS LOCI	100 HETEROZYGOUS LOCI
Recessive lethal mutants ($R_A=1$; $R_a=0$) $u=10^{-5}$	8,300	16,000
Recessive subviable mutants ($R_A=1$; $R_a=.9$) $\mu=10^{-5}$	2,500	5,000
Heterotic gene pairs:		
$R_A=.8$; $R_a=.6$	98	196
$R_A=.4$; $R_a=.2$	67	135

Thus it becomes evident that in order to build up an efficient heterotic mechanism by means of mutation to subviable recessive alleles one would require mutation at practically more than every locus in the corn plant.

On the other hand, the hypothesis of the heterotic subviable loci does not involve any such difficulty since a very small fraction of the total of loci in maize are sufficient to explain heterosis of any individual character.

THE RESULTS OF INBREEDING UNDER THE TWO ALTERNATIVE SYSTEMS

We have to establish a formula which will allow us to calculate the genotypic frequencies in populations that are subjected to close inbreeding. The results, to be expected when individuals carrying recessive lethals are selfed, have been discussed in great detail during the last forty years, but in addition we require a new and special formula which will permit us to extend the discussion and to include both subviable recessive genes and heterotic gene pairs. The necessary formulas have been developed by BRIEGER (1948a, 1948b). To simplify the question we shall take only into consideration the initial frequencies of the three genotypes AA, Aa and aa and the survival values R_A and R_a of the two homozygotes, while the survival value of the heterozygotes is taken as equal to one and used as basic value for comparison. We may omit the possibility of any new mutation, since with the small mutation rates ordinarily found only insignificant changes can occur. The formula is valid only for populations, but not for selected groups of inbred pedigree lines.

Starting from the initial frequencies u, v and w for the three genotypes we obtain in the mth generation of selfing, the following frequencies:

$$\begin{aligned} AA:&\quad 4\cdot u\cdot R_A^m + v\cdot\left\{1+(2R_A)+(2R_A)^2+\cdots+(2R_A)^{m-1}\right\}\cdot R_{Av}\\ Aa:&\quad 2v \qquad\qquad (7)\\ aa:&\quad 4\cdot w\cdot R_a^m + v\cdot\left\{1+(2R_a)+(2R_a)^2+\cdots+(2R_a)^{m-1}\right\}\cdot R_{av}. \end{aligned}$$

The first term in the formula for frequencies of homozygotes can be omitted since its value will be comparatively small, except in rare cases where the initial frequency v, of heterozygotes, is much smaller than u and w.

The second term has some very interesting mathematical properties:

If the survival value for any of the homozygotes is equal to or larger than .5,

the exponential series will increase indefinitely and thus all heterozygotes will tend to disappear rapidly from the population. If in this case R_A should be larger than R_a, the frequency of the corresponding homozygote will increase more rapidly and reach infinity before that of the other homozygote. Consequently only the homozygotes with higher survival value will remain in the population. The same will occur and much more rapidly when only one of the survival values is larger and the other smaller than .5.

When both survival values of homozygotes are smaller than .5, all the exponential series will reach a finite value, and thus both heterozygotes and homozygotes will remain present in the population at equilibrium. I repeat from another publication (BRIEGER 1948) data (table 4, lower part) which contain the expected frequencies for all three genotypes at equilibrium. These values are calculated easily since the exponential series in formula (8) may give rather simple values such as: 5 for $R=.4$; 2.5 for $R=.3$; 1.66 for $R=.2$, and 1.25 for $R=1$.

Thus there are two quite different alternatives of population equilibrium under selfing: If one or both survival values of homozygotes are larger than one half, only the homozygotes with the higher survival value will be preserved. If both these survival values are below one half, the population will always contain all three genotypes, the two homozygotes and the heterozygotes (table 4, lower part). The second alternative is of special importance, because it shows that current belief is wrong in as far as selfing does not always lead automatically to complete homozygosis.

The approach to equilibrium will be gradual in selfed populations with large numbers of individuals. In most inbreeding experiments, however, the situation will be different. If we establish pedigree lines no compensation is possible in later generations for the accidental selection of a given individual as parent of the next generation. If this individual should contain more homozygous loci than expected for the mean of its generation, no return to a higher degree of heterozygosis is possible. If the chosen individual was more heterozygotic than expected, this corresponds, on the other hand, to a return to frequencies of a previous generation and thus a slowing down of inbreeding speed. Thus genetic drift in small inbreeding units may either slow down or speed up the process of reaching final equilibrium and of obtaining homozygosis. It may also lead to establishing genotypic proportions which are quite different from the expected mean. Selfed pedigree lines will tend on the whole to establish a higher degree of homozygosis more quickly than would be expected in large inbreeding populations where different pedigree lines are not kept separated.

We are now in a position to discuss whether or not our two alternative explanations of heterosis, the dominance hypothesis and the hypothesis of heterotic gene interaction, are in accord with theoretical expectation. In order to simplify matters we shall limit the discussion by assuming that the individuals are at the beginning completely heterozygous for ten pairs of factors. This is a considerable simplification since normally the number of loci involved in corn will be much higher.

We shall begin with the *dominance hypothesis*. The frequency of hetero-

zygotes expected in the first, third and sixth generation of an inbred population are illustrated in figure 3, and it is evident that the survival value of the recessive homozygotes has very little influence on the final results. In the first inbred generation, the individuals contain on the average more than half of their loci in a heterozygous condition, and hardly any individual contains less than three or more than eight or nine heterozygous loci of the ten loci in consideration. However, in the sixth generation, between 70 and 80 percent of individuals are completely homozygous, and almost no individual should contain more than two out of the ten loci in the heterozygous state.

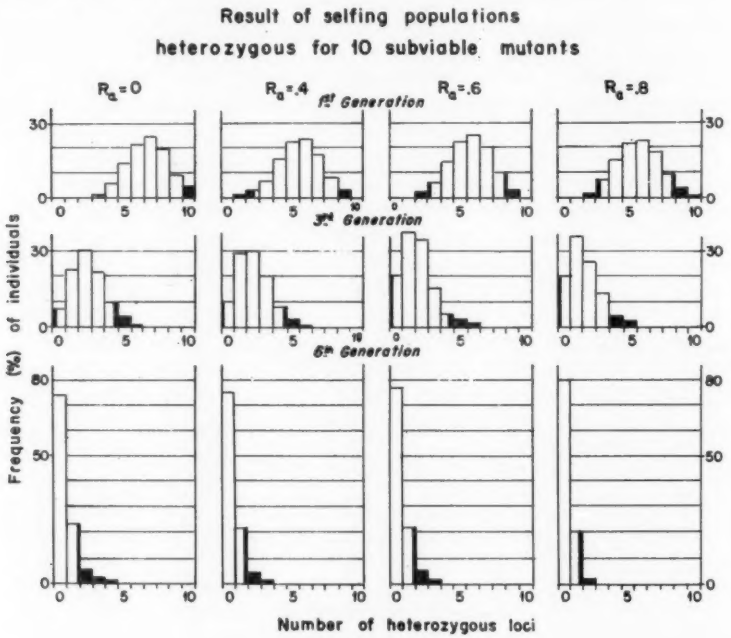


FIGURE 3

The influence of survival values becomes somewhat more pronounced if we take into consideration the number of dominant or recessive homozygous loci per individual plant. In the third inbred generation we still expect a considerable number of homozygous subviables while after the sixth generation they tend to disappear except when survival indexes are quite near the value one.

If we are dealing with inbred pedigree lines instead of populations, it is clear that on the whole they also should tend to become homozygous and contain at least some subviable recessive characters, unless we are always able to select the most vigorous of the inbred lines in such a way that they contain almost only dominant factors for normal viability and fertility.

All the authors who have studied the problem of inbreeding during the last forty years, have already stated that the dominance hypothesis leads us to postulate that inbred families should be nearly homozygous from about the sixth selfing onward, and we have just shown that this rule holds true whether we are dealing with lethal genes or with subviable mutants causing only a rather slight reduction of survival values. However, it seems to me that it has been taken too frequently for granted that such a high degree of homozygosis is really attained. Uniformity or homogeneity in a population or an inbred line is not necessarily a proof for homozygosis, since balanced heterozygous popula-

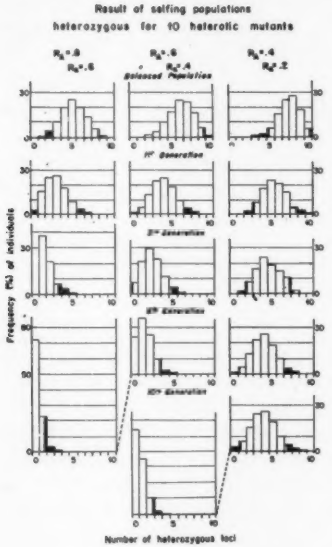


FIGURE 4

tions can easily also be quite uniform. We shall come back to this important point again later on.

Now we shall discuss the behavior of inbred populations carrying *heterotic gene pairs*. Figure 4 shows the existence of pronounced different results in accordance with the values of survival indexes. If the survival values of both homozygotes are rather high the situation does not differ very much from that just described for subviable recessives. In the sixth inbred generation about 70 percent of all individuals should be completely homozygous and very few individuals should be heterozygous for more than one locus. If both survival values are close to .5, with at least one above this limit, the final result will again be the same, except that, many more generations will be required, and even in the tenth inbred generation we will have only about 50 percent homozygous individuals, while the other half of the population will still be heterozygous for one or two of ten loci. Finally, if both survival indexes are below .5,

the picture changes completely, and no complete homozygotes are expected even in the tenth inbred generation. The mean number of heterozygous loci will be about four out of the ten loci present and the maximum may be seven out of ten loci.

Finally, the frequency of loci which should become homozygous for the less viable alleles is considerably higher than the values for homozygous subviable recessives. In the three cases of heterotic genes under discussion only about 10 to 20 percent of all individuals may become completely homozygous for the genes with a higher survival value.

In short, we may say that factor pairs with a heterotic gene interaction: (a) will tend to maintain a higher degree of heterozygosis for a longer period, and (b) do not favor the appearance of individuals which are completely homozygous for all the genes with the higher survival value. Inbreeding in pedigree lines instead of in populations will somewhat alter the final results, without changing anything fundamentally.

The hypothesis of heterotic gene interaction is thus also in accord with the facts generally observed, and we now have to decide which of the two alternative hypotheses gives a better and more satisfactory explanation of all facts.

In the first instance, I want to repeat my doubt in the current belief of attaining complete homozygosity by inbreeding, as required only under the dominant hypothesis. In fact, it seems to me far from being proved experimentally that inbreeding yields an almost complete homozygosis in about six generations, and moreover there are some clear indications that this is at least not always the case. For instance, several years ago we received from DR. M. M. RHOADES a highly inbred line of "good pachytene" maize and expected it to be highly homozygous. The line when first grown in Brazil was very weak, but by using the best ears obtained from selfing we were able to improve the climatic adaptation in about three generations. Such an adaptive change is, of course, only possible, if there still existed a sufficient number of heterozygous loci, and thus our observations prove that the inbred line still contained "hidden" heterozygosity. By growing the inbred line in a completely different climate, we evidently changed the adaptive value of many genes and thus established a new and different level for final equilibrium. We started a new selective trend which could only have been effective if sufficient heterozygosity was still present.

Next we shall consider a surprising situation present in indigenous maize varieties. Since the Indians have grown maize many generations in small plots or populations, we should expect a high degree of inbreeding. Under the dominance hypothesis a high degree of homozygosis should have been attained, while the theory of heterotic gene interaction hypothesis would permit the maintenance of a considerable degree of heterozygosis, if survival values for homozygotes should be rather low. My extensive studies on South-American indigenous maize have definitely shown that the latter alternative is correct. The degree of heterozygosis must be quite considerable, since marked changes may be produced by a few generations of selection. Furthermore, the reduction of vigor even in the first inbred generation is so pronounced

that numerous indigenous types cannot be maintained after two or three selfings, because no ears with good kernels are produced. We must assume that the Indian plant breeder, by always selecting the best ears and the strongest plants succeeded in attaining an extreme degree of heterozygosity at heterotic loci. At the same time, it may be mentioned that by selecting typical ears of their preferred varieties, they also achieved a genetic drift in favor of uniformity of type and a high degree of homozygosis at certain other loci, than those responsible for "vigor."

I may also mention that by a special breeding technique I have been able to select a new type of sweet corn adapted to the Brazilian subtropical conditions. It represents a balanced population, in the same way as the indigenous varieties: maintaining automatically both its vigor and heterozygosity for heterotic gene pairs, and uniformity for type. When a plant of such a population is selfed there is pronounced reduction of vigor in the first generation.

The residual heterozygosity of inbred lines, the existence of balanced heterotic populations in indigenous South-American maize and the possibility of actually obtaining such balanced populations by breeding are facts which are definitely not in accord with the dominance hypothesis, but can be readily explained by the alternative hypothesis of heterotic gene interaction. Thus again we reach the same conclusion as in the preceding chapter: only the second of the two hypotheses is completely in accord with the observed facts.

REFERENCES TO BREEDING PROCEDURE

There is no doubt that the method called "hybrid corn" has given excellent results in the United States, but the results obtained by this method elsewhere are far from satisfactory. There is at least one rather simple explanation for this divergence of results. The number of research workers, Experiment Stations and farmers engaged in the breeding program in the United States is in excess of and not even comparable to the very low intensity of the work carried out in other countries. There, isolated geneticists try to achieve practical results with limited numbers of inbred lines, obtained from limited material from a few sources only. However, it may well be that other and more fundamental reasons are involved as well. It is a known fact that the North American dent corns were not directly derived from any existing populations but from variety hybrids. In the original hybrids between the tall southern dent or "gourd seed" varieties and the smaller northeastern little flints, a large amount of factor interaction between size factors must have been involved. Therefore, the attempts to select again by simple mass selection in crossbred populations for a uniform dent type may have created a situation quite different from that existing in old indigenous balanced populations. It seems to me to be rather difficult to judge the results of unscientific mass selection, carried out between about 1830 and 1910. Anderson (1945) reports that most of the modern lines of inbred dent corn in the United States can be traced back to a few original selections, mainly to Reid's Yellow Dent and to Lancaster Sure-cropper. The vast majority of the practical breeders of the last century have thus failed in producing a good new variety. At the same

time, it seems to me very doubtful that these selections may have again established balanced heterotic populations, which are characterized by their sensitivity to any degree of inbreeding. The basic technique in modern corn breeding is to select lines which can stand a good deal of inbreeding and which, therefore, require high values for the survival index of homozygotes. Thus the basic genetic element which enables us to establish a balanced heterotic population is intentionally eliminated from the material by the maintenance of only such inbred lines that still remain rather productive.

Since the US-dent varieties are to a large extent derived from intravarietal hybridization, the hybrid corn technique is probably quite appropriate for them, and represents the best and most rapid practical solution for the North American plant breeder. However, it seems to me very doubtful whether the same method will give equally good results in other countries, such as the South-American ones, where we have at our disposal local or indigenous balanced populations, containing a large number of genes with a low survival index when homozygous. In fact, it may be more expedient instead to establish new and improved balanced populations and to study in detail to what extent balanced populations of maize may not be only the first but also the final solution of the breeding problem, at least in South and Central America where we have generally the basic gene material at our disposal.

The breeding procedure in establishing such balanced populations differs in several points from the ordinary technique. We must inbreed for about three generations in order to eliminate recessive undesirable characters and to increase homogeneity of non-heterotic loci. During this inbreeding phase, preference should be given to lines which are less resistant to inbreeding since they will contain heterotic alleles for low survival values in homozygotes necessary for building up the balanced population. In the second phase of the work, we must carry out repeated and multiple crosses eliminating all lines with low combining ability. After another period of about three generations of continuing crossing in selecting, the remaining multiple hybrids should be thrown together to form now one breeding unit. If the procedure has been carried out successfully, a new balanced population will be obtained.

SUMMARY

1. The use of the term heterosis is discussed. It is suggested that one should apply this term whenever the mean of hybrids for any quantitative character is higher than that of descendants, obtained by any form of inbreeding. Hybrids which are heterotic in this sense, may be superior, equal to, or inferior to their own parents. Furthermore, heterosis does not affect an individual as a whole, but only its separate characters.

2. Two fundamentally different genetic formulas have been proposed during the last forty years in explanation of heterosis: The dominance hypothesis is based on the assumption that heterotic hybrids are heterozygous for recessive subviable mutant genes, while the alternative explanation postulates the existence of loci containing special "heterotic" alleles which when heterozygous increase vigor. The linkage hypothesis represents an intermediary solution

between these alternatives and was intended to overcome certain difficulties. The evidence presented in the literature up to now gives no conclusive proof in favor of either of the hypotheses.

3. Applying the principles of population genetics to the problem of the evolution of heterotic gene mechanisms, we were able to show that there exists no effective breeding system which would tend to accumulate a sufficient number of recessive subviable or lethal mutants, as required by the dominance hypothesis. But there will be a rapid accumulation of heterotic mutations, which does not depend upon the mutation rate.

4. Using the number of heterozygous loci present in a population as a measure for the efficiency of a genic system, it is evident that heterotic gene systems are much superior to systems based on recessive subviable or lethal mutations. In order to accumulate an average of 100 heterozygous loci per individual in a population, we require (a) from 100 to 200 mutable loci, producing mutations to heterotic gene pairs, (b) 5,000 loci which may mutate at a rate of 10^{-5} to recessive subviable genes with survival values of the high order of .9 or finally (c) over 15,000 loci which may mutate at the rate of 10^{-5} to lethal mutant genes.

5. Facts are cited to show that the dominance hypothesis is not sufficient to explain all results obtained by inbreeding. The hypothesis of heterotic gene interaction on the other hand is again capable of explaining in a satisfactory manner all known facts.

6. A certain number of recessive subviable or lethal mutations tend to accumulate in any random mating population, and thus the appearance of the corresponding homozygotes after inbreeding is not a cause, but rather a consequence of the breeding system.

7. Two alternative methods of maize breeding are mentioned. They may be characterized by the terms "hybrid corn technique" and "balanced population technique." Their advantages and disadvantages are briefly discussed.

LITERATURE CITED

ANDERSON, E., 1945 The sources of effective germ plasma. Ann. Missouri Bot. Garden **31**: 355–361.

BRIEGER, F. G., 1930 Selbst- und Kreuzungssterilität. xi+395 pp. Berlin: Julius Springer.

1944 Estudos experimentais sobre origiem do milho. An. Esc. Sup. Agric. **2**: 225–278.

1948a Nocoes sobre a genetica em populacoes. An. Esc. Sup. Agric. in press.

1948b Heterosis in Population Genetics and Evolution. Palanza Meeting July 1948 (in press).

BRUCE, A. B., 1910 The Mendelian theory of heredity and the augmentation of vigor. Science **32**: 672–628.

COLLINS, G. N., 1918 Intolerance of maize to self-fertilization. J. Wash. Acad. Sci. **9**: 309–312.

CORRENS, C., 1920 Pathologie und Vererbung bei Pflanzen und einige Schlüsse daraus für die vergleichende Pathologie. Med. Klinik **16**: 364–369.

CROW, J. F., 1948 Alternative hypotheses of hybrid vigor. Genetics **33**: 477–487.

DOBZHANSKY, TH., 1943 Genetics of natural populations. IX. Temporal changes in the composition of populations of *Drosophila pseudoobscura*. Genetics **28**: 62–86.

1947a A directional change in the genetic constitution of a natural population of *Drosophila pseudoobscura*. Heredity **1**: 53–64.

1947b Genetics of natural populations. XIV. A response of certain gene arrangements in the

third chromosome of *Drosophila pseudoobscura* to natural selection. Genetics **32:** 142–160.

1947c Adaptive changes induced by natural selection in wild populations of Drosophila. Evolution **1:** 1–16.

DUNN, L. C., and ERNST CASPARI, 1945 A case of neighboring loci with similar effects. Genetics **30:** 543–568.

EAST, E. M., 1936 Heterosis. Genetics **21:** 375–397.

EAST, E. M., and H. K. HAYES, 1912 Heterozygosis in evolution and in plant breeding. Bull. U. S. Dept. Agric. **243:** 7–58.

GUSTAFSON, A., 1946a The effect of heterozygosity on variability and vigour. Hereditas **32:** 263–286.

1946b The advantageous effect of deleterious mutations. Hereditas **33:** 573–575.

HALDANE, J. B. S., 1926 A mathematical theory of natural and artificial selection. III. Trans. Camb. phil. Soc. **23:** 363–372.

1927 Mathematical theory of natural and artificial selection. V. Selection and mutation. Proc. Camb. phil. Soc. **23:** 838–844.

1937 The effect of variation on fitness. Amer. Nat. **71:** 337–349.

HULL, F. H., 1945 Recurrent selection and specific combining ability in corn. J. Amer. Soc. Agron. **37:** 134–145.

1946 Overdominance and corn breeding where hybrid seed is not feasible. J. Amer. Soc. Agron. **38:** 1100–1103.

1948 Evidence of overdominance in yield of corn. Genetics **33:** 110.

JONES, D. F., 1917 Dominance of linked factors as a mean of accounting for heterosis. Genetics **2:** 466–479.

1944 Equilibrium in gene materials. Proc. nat. Acad. Sci., Wash. **30:** 82–87.

1945 Heterosis resulting from degenerative changes. Genetics **30:** 527–542.

KALMUS, H., 1945 Adaptative and selective response of a population of *Drosophila melanogaster*, containing *e* and e^+, to differences in temperature, humidity and to selection for developmental speed. J. Genet. **47:** 58–63.

KEEBLE, F. and C. PELLEW, 1910 The mode of inheritance of stature and of time of flowering in peas (*Pisum sativum*). J. Genet. **1:** 47–56.

KERR, W. E., 1950 Genetic determination of castes in the genus Melipona. Genetics **35:** 143–152.

L'HERITIER, P., and G. TEISSIER, 1933 Étude d'une population de Drosophiles en équilibre. C. R. Acad. Sci. (Paris) **197:** 1765–1767.

1934 Sur quelques facteurs du succès dans la concurrence larvaire chez *Drosophila melanogaster*. C. R. Soc. Biol. (Paris) **116:** 306–308.

1937a Élimination des formes mutantes dans les populations de Drosophiles. Cas des Drosophiles "bar." C. R. Soc. Biol. (Paris) **124:** 880–882.

1937b Élimination des formes mutantes dans les populations de Drosophiles. Cas des Drosophiles "ebony." C. R. Soc. Biol. (Paris) **124:** 882–884.

LINDSTROM, E. W., 1935 Genetic experiments on hybrid vigor in maize. Amer. Nat. **69:** 311–322.

MÜNTZING, 1946 On the causes of inbreeding degeneration. Arch. J. Klaus. Stiftg. Suppl. to vol. **20:** 153–163.

RASMUSSON, J., 1933 A contribution to the theory of quantitative character inheritance. Hereditas **18:** 245–261.

RICHEY, F. D., 1945 Bruce's explanation of hybrid vigor. J. Hered. **36:** 243–244.

1946 Hybrid vigor and corn breeding. Amer. Soc. Agric. **38:** 832–841.

SHULL, G. H., 1911a Hybridization methods in corn breeding. Amer. Breeders Mag. **1:** 98–107.

1911b The genotypes of maize. Amer. Nat. **45:** 234–252.

1922 Ueber die Heterozygotie mit Rücksicht auf den praktischen Züchtungserfolg. Beitr. Pflanzenz. **5:** 134–152.

1948 What is heterosis? Genetics **33:** 439–446.

SINGLETON, W. R., 1943a Breeding behavior of C 30, a diminitive P 39 mutant whose hybrids show increased vigor. Genetics **28:** 89.

1943b Hybrid vigor in the intra-bred cross $P_{39} \times C_{39}$ in maize. Genetics Soc. Amer. Rec. **12:** 52–53.

SPRAGUE, G. F., 1946 The experimental basis for hybrid maize. Biol. Rev. **21:** 101–120.

STERN, C., 1948a The effect of change in quantity, combination and position of genes. Science **108:** 615–621.

1948b Negative heterosis and decreased effectiveness of alleles in heterozygotes. Genetics **33:** 215–219.

STERN C., and E. W. SCHAEFFER, 1943 On wild-type iso-alleles in *Drosophila melanogaster*. Proc. nat. Acad. Sci. **29:** 361–367.

STERN, C., R. H. MCKNIGHT and M. KODANI, 1946 The phenotype of homozygotes and hemizygotes of position alleles and of heterozygotes between alleles in normal and translocated position. Genetics **31:** 598–619.

STUBBE, H. and K. PIRSCHLE, 1940 Ueber einen monogen bedingten Fall von Heterosis bei *Antirrhinum majus*. Ber. dtsch. bot. Ges. **58:** 546–558.

TEISSIER, G., 1942 Vitalité et fecondité relatives de diverses combinations genetiques comportantes un gène lethal chez la Drosophile. C. R. Soc. (Paris) **214:** 676–677.

1944 Équilibre des gènes lethaux dans les populations stationnaires panmictiques. Rev. sci. (Paris) **82:** 145–159.

1947a Variation de la fréquence du gène sepia dans une population stationnaire de Drosophiles. C. R. Soc. Biol. (Paris) **224:** 676–677.

1947b Variation de la fréquence du gène ebony dans une population stationnaire de Drosophiles. Comp. Rend. Soc. Biol. (Paris) **224:** 1788–1789.

WHALEY, W. G., 1944 Heterosis. Bot. Rev. **10:** 461–498.

WRIGHT, S., 1931 Evolution in Mendelian populations. Genetics **16:** 97–159.

1947 On the genetics of several types of silvering in the guinea pig. Genetics **32:** 115–141.

WRIGHT, S. and TH. DOBZHANSKY, 1946 Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. Genetics **31:** 125–156.

CHROMOSOME SEGREGATION IN TRANSLOCATIONS INVOLVING CHROMOSOME 6 IN MAIZE[1]

C. R. BURNHAM

University of Minnesota, St. Paul, Minnesota

Received December 30, 1949

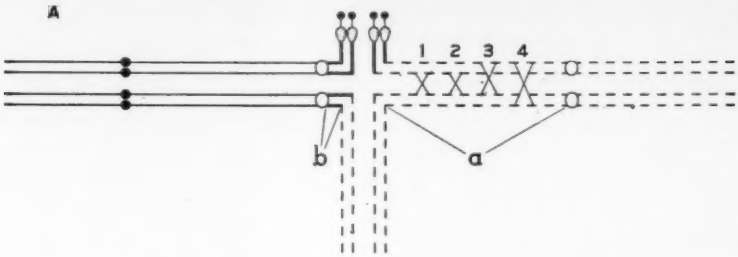
ONE of the unsolved problems concerning translocations deals with chromosome segregation. Why is it mostly alternate in Oenothera, while in maize both alternate and adjacent segregations occur? Maize translocation heterozygotes involving chromosome 6 which carries a nucleolar organizer region are useful for such a study, since certain of the types of segregation result in recognizably different microspore quartets (McCLINTOCK 1934, 1945). The results of a study of chromosome segregation using 27 translocations with breakages at different loci in chromosome 6 are presented in this paper, the theoretical results first, followed by the experimental data.

THEORETICAL RESULTS IN THE SPORE QUARTETS AND POLLEN OF TRANSLOCATION HETEROZYGOTES

Considering only the theoretically possible 2-2 segregations from the complex of four chromosomes (fig. 1A) alternate or adjacent chromosomes may pass to the same pole, there being two types of adjacents, designated by McCLINTOCK (1945) as: adjacent 1 in which homologous centromeres pass to opposite poles at division I (disjunction of homologous centromeres, nondisjunction of the translocated pieces) and adjacent 2 in which they pass to the same pole (nondisjunction of homologous centromeres, disjunction of the translocated pieces). These three types of segregation may be thought of also as resulting from different planes of separation at the first division, alternate and adjacent 1 being along a plane which separates homologous centromeres and adjacent 2 along a plane at right angles to this. When the segregation involves nondisjunction of the nucleolar organizer regions, two types of spores result, one having two organizers and potentially two nucleoli which may fuse, the other type having no organizer and consequently scattered small bodies of nucleolar material, referred to in this paper as a "diffuse" spore type. In maize the spores remain in quartets for a time after completion of meiosis, and with the proper acetocarmine staining any spore quartets differing in their nucleolar makeup may be recognized. It is possible also to distinguish the two division planes, since a cell division follows the first meiotic division.

The expected results of these three kinds of segregation fall into three main

[1] Contribution from the Division of Agronomy and Plant Genetics, UNIVERSITY OF MINNESOTA, St. Paul, Minn. Paper No. 2479 of the Scientific Journal Series, MINNESOTA AGRICULTURAL EXPERIMENT STATION. Assistance was furnished from research funds of the UNIVERSITY OF MINNESOTA GRADUATE SCHOOL. A sabbatical leave, together with a GOSNEY FELLOWSHIP at the CALIFORNIA INSTITUTE OF TECHNOLOGY (1947–48), made its completion possible. I wish to acknowledge the assistance of GERTRUD JOACHIM, C. H. LI, and H. R. HIGHKIN in gathering the pollen sterility and quartet data. I am indebted to C. M. ARNDT for drawing figures 1A and B.



B

PHENOTYPES AND FUNCTIONAL BEHAVIOR

EVENTS IN INTERSTITIAL SEGMENTS	CHROMATID PAIRS AFTER CROSSING OVER	KIND OF SEGREGATION	NO DIFFUSE	TWO DIFFUSE	ONE DIFFUSE
NO CROSSOVER IN *a* OR *b*	•• •• / −− −−	ALTERNATE ADJACENT 1 ADJACENT 2		1	
SINGLE CROSSOVER IN *a* OR *b*	•• •− / −− •−	ALTERNATE ADJACENT 1 ADJACENT 2		1	1
SINGLE CROSSOVER IN *a* AND *b*	•− •− / •− •−	ALTERNATE ADJACENT 1 ADJACENT 2		1	2 2 2
DOUBLES – *a* OR *b*					
2 STRAND – 1, 2	•• •• / −− −−	SAME AS NO CROSSOVER			
3 STRAND – 1, 3	•• •− / −− •−	SAME AS SINGLE CROSSOVER			
4 STRAND – 1, 4	•• −− / −− ••	ALTERNATE		1	
		ADJACENT 1 ADJACENT 2		1	

FIGURE 1A. Pachytene diagram of a 5–6 translocation heterozygote with the break in chromosome 6 (solid line) in the short arm, that in 5 (dotted line) in the long arm, *b* and *a* being the interstitial segments in the respective chromosomes. At the short arm end of chromosome 6 are the satellite and next to it the nucleolar organizer region (stippled). The clear circles represent the centromeres, the dense ones the pycnotic knobs.

FIGURE 1B. The spore quartet types and their frequencies resulting from various events (crossovers or non crossovers) in the interstitial segments in figure 1A. The second column shows the nucleolar organizer constitutions of the resulting chromatid pairs (with sister centromeres) before orientation for Division I; • and — indicate chromatids with and without the nucleolar organizer respectively. The shaded spores in the resulting quartets are normal, the empty ones abortive due to chromosomal deficiency. The first division plane is indicated by the wider separation. The two abnormal spores in the first quartet type in the "one diffuse" class, and the two diffuse-nucleolate spores in the second quartet type in the "two diffuse" class should be by chance contiguous but on opposite sides of the first division plane as frequently as in the diagonal position shown.

groups depending on the position of the breakage point in chromosome 6: (1) those with the break in the short arm between the centromere and the nucleolar organizer, (2) those with the break in the long arm or in the satellite, and (3) those with the break in the nucleolar organizer. Segregation of two chromosomes to each pole will be considered first, then the results from 3-1 segregation.

Group 1, those with one break in the short arm of chromosome 6 between the centromere and the nucleolar organizer.

The expected results for this group have been presented (BURNHAM 1949), but are summarized here in figure 1B. Three main types of spore quartets may be recognized cytologically based on the number of spores with "diffuse" nucleolar material, i.e. "no diffuse," "two diffuse," and "one diffuse." The first division is assumed to be reductional for homologous centromeres (in adjacent 2 segregations the first division is nondisjunctional for them but the second division is again equational). By referring to figure 1A and to column 2 in figure 1B, the kinds of quartets expected from each type of segregation, with and without crossing over[2] in the interstitial segments, may be seen to be those shown in the last three columns of figure 1B. For example with no crossing over in the interstitial segments, at the end of the first division following alternate segregation one pair of chromatids at each pole has two nucleolar organizers (indicated by dots) and one pair has no organizers (indicated by dashes): . At the second division, one chromatid from each of the two pairs passes to the same pole, resulting in a quartet of normal functional spores (indicated by shading in fig. 1B) with one nucleolus each: . Adjacent 2 segregation results in a quartet indistinguishable in appearance from this (one nucleolus in each spore) but which differs from it in having spores all of which are deficient and expected to abort. Adjacent 1 segregation results in the quartet type which has two "diffuse" spores, a "two diffuse" quartet. With no crossing over in the interstitial segments, the difference between the observed pollen abortion and the amount predicted from the frequency of "two diffuse" quartets should indicate the frequency of adjacent 2 segregation and hence the frequencies of the three types of segregation could be determined.

A single crossover in either interstitial segment, followed either by alternate or adjacent 1 segregation results in the third recognizable spore quartet type having two spores with one nucleolus each (functional), one with potentially two nucleoli, and one with diffuse nucleolar material, the latter two being on opposite sides of the first division plane and abortive (fig. 1B). This will be referred to as a "one diffuse" or a crossover type quartet. The segregation in it has been referred to in certain Oenothera and Pisum studies on translocations as "half disjunction." A high frequency of such crossovers in a species having mainly alternate segregation in rings should result in sterility approaching 50 percent. If adjacent 2 segregation followed such a crossover, a "no diffuse" as well as this "one diffuse" type would be expected, in both of which all four spores would be abortive. However, if chromosomes that crossover pass to opposite poles, as evidence reported here and by MCCLINTOCK (1945) indicates, this type of segregation would not occur. In that case, in a translocation with only one interstitial segment that is short enough to preclude

[2] Throughout this paper the terms "crossing over" or "crossovers" refer to the cytological process, contrasted with "genetic crossing over" or "recovered crossovers" for the observed genetic result. Unless stated otherwise, the crossovers referred to are those occurring in interstitial segments.

double crossovers it would be possible to measure the frequency of single crossovers in this segment, and of the different kinds of segregation from the non crossover meiocytes.

Simultaneous single crossovers in the two interstitial segments result in the three types of quartets from each kind of segregation (fig. 1B). Translocations in which this is frequent would not be as satisfactory for an analysis of chromosome segregation. Note that the two abnormal spores in the "crossover" type and the two "diffuse" spores in the "two diffuse" quartet are on the same side and on opposite sides, respectively, of the first division plane; whereas the reverse is true for single crossing over in one segment and for no crossing over.

The expected results from double crossing over in either region a or b are also shown in figure 1B. The 2-strand doubles give the same quartet types as with no crossing over, and the 3-strand doubles give the same types as does a single crossover. Following a 4-strand double, adjacent 1 segregation gives rise to a normal quartet with functional spores while alternate segregation results in a quartet having two spores with diffuse nucleoli (all four spores abortive), just the reverse of those following no crossing over. Only if alternate and adjacent 1 segregations following 4-strand double crossing over are equally frequent would the frequency of quartets containing two spores with diffuse nucleoli still be an accurate measure of adjacent 1 segregation. Although adjacent 2 segregation may not be expected following the 2- and 4-strand doubles, if it did occur it would result in normal-appearing quartets with four abortive spores and hence would be included in any observed difference between predicted and observed sterility.

A very important point, previously overlooked, has been presented by LAMM (1948) based on TOMETORP'S unpublished analysis (called to my attention by DR. H. H. KRAMER as this was being completed), (see also HANSON and KRAMER 1949) i.e. that the two crossover chromatids resulting from a single crossover in the interstitial segment are carried by the abortive spores when the segregation is alternate; and are carried by the normal spores only when it is adjacent 1. Double crossover chromatids following 2-strand and 3-strand doubles, and single crossover chromatids following 4-strand doubles may be recovered when the segregation is alternate. In species having mostly alternate segregation the observed genetic crossing over should be greatly reduced. In corn where alternate and adjacent 1 segregations are probably equally frequent following the crossovers, reduction in observed recombination would be expected from that source.

In presenting the data, it is assumed that the first division is reductional at the centromere. The evidence from other sources makes this probable, although certain infrequent quartet types to be described later could be explained by rare equational separation.

Where the breaks are fairly close to the centromeres, non-homologous pairing may separate homologous centromeres. This would not necessarily result in equational separation of these centromeres at division 1, but might affect the action of the centromeres on orientation from those figures.

It should also be noted that in the quartets containing functionally normal spores a ratio of 1 with the standard normal:1 with the translocation chromosome complex is expected.

Group 2. Translocations with one break in the long arm or in the satellite of chromosome 6.

In plants heterozygous for a translocation in which the break in chromosome 6 was in the long arm, the pachytene configuration is represented in figure 2; c and d indicating the interstitial segments. In this type of translocation, a study of the diagram will show that adjacent 2 segregation results in

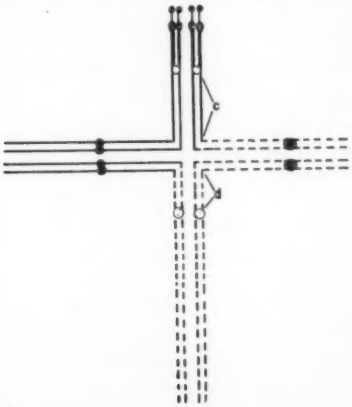


FIGURE 2. Pachytene diagram of a 5–6 translocation heterozygote with the breaks in 6 (solid line) and 5 (dotted line) in the long arms, c and d being the interstitial segments in the respective chromosomes. Adjacent 2 segregation (when homologous centromeres pass to the same pole) results in the "two diffuse" type, alternate or adjacent 1 segregation results in the "no diffuse" type, with or without crossing over in the interstitial segments.

"two diffuse" quartets, while alternate and adjacent 1 segregations result in "no diffuse" quartets, with or without crossing over in either or both interstitial segments. The frequency of "two diffuse" quartets is the frequency of adjacent 2 segregation. Single crossovers and 3-strand doubles again result in "half disjunction" quartets in which two spores are normal and two abortive, but they have one nucleolus each and cannot be recognized cytologically. If the interstitial regions are so short that little or no crossing over occurs in them, the excess of observed over predicted pollen abortion would measure the frequency of adjacent 1 segregation (quartets which appear normal but in which all four spores abort). The remaining normal, i.e. "no diffuse," quartets would result from alternate segregation. In those having either or both interstitial segments long, only if there is independent information on the frequency of single and multiple crossing over in them can the frequency of adjacent 1 and alternate segregations be estimated. Translocations of this type, group 2, are useful since in all of them the frequency of adjacent 2

segregation can be determined. No assumptions regarding the kind of segregation following crossing over are necessary, the only difficulty coming from 3-1 segregation to be discussed later.

The problem of segregation frequencies might be solved also by cytological determinations of the relative frequencies of open and zigzag rings at metaphase if it could be assumed that only the zigzag rings lead to alternate segregation. However, KOLLER (1944) has reported in mice that the zigzag rings may be oriented to give adjacent segregation. If differences in morphology are great enough, the two types of adjacents may be recognized at early anaphase I. Such a cytological study would not be complete unless it were possible to recognize those figures in which crossing over had occurred in an interstitial segment.

For *translocations with one break in the satellite of chromosome 6*, the quartet types are the same as for the preceding ones in which the break in chromosome 6 was in the long arm. One difference in this group is that spores deficient only for a portion of the satellite are not abortive (BURNHAM 1932b), hence allowance must be made for this in comparing observed with predicted pollen abortion.

Group 3. Translocations with one break in the nucleolus organizer region of chromosome 6.

In the translocations with the break in chromosome 6 in the nucleolus organizer region, if both pieces of that region (the translocated and the remaining piece) retain the nucleolar organizing function there will be a potential total of six nucleoli in the normal quartet, only two of which should be normal in size. The nucleoli organized by the pieces of the organizer region may be of similar size or unequal (MCCLINTOCK 1934). Various combinations of these in the spores of a quartet are possible with different segregations and with crossing over. Since fusion of these nucleoli often occurs, an accurate determination of the relative frequencies of different segregations would be difficult or impossible from the quartet study. A quartet in which one spore has diffuse nucleoli is theoretically possible if adjacent 2 segregation occurs following crossing over in an interstitial segment. A cytological determination of the frequencies of zig-zag and open rings may be the best means of studying segregation in translocations belonging to this group. MCCLINTOCK (1934) has reported the results for one such translocation (*T6–9a*) by identifying cytologically the chromosome constitutions of the microspores.

Spore quartet types following 3-1 disjunction of chromosomes from the ring. A 3-1 segregation of the chromosomes in the ring may result in a "two diffuse" or a "no diffuse" quartet, depending on whether two or only one of the three chromosomes going to one pole carries a nucleolar organizer. In either case, two spores are normal, two abortive. If the different kinds of 3-1 segregation were at random, these two types would be equally frequent and the errors in the predicted sterility would balance each other. As a check on possible sources of error, it may be important to determine the frequency with which "two diffuse" quartets may be expected from 3-1 segregation.

EXPERIMENTAL METHODS

Frequency counts of the different types of quartets based on their nucleolar makeup were used to measure directly the frequencies of certain kinds of chromosome segregation. The frequency of other segregations was determined by comparing the observed pollen abortion with that predicted from the frequencies of quartets whose nucleolar makeup indicated abortive spores.

Pollen abortion counts were made by teasing about a third of an anther into a small drop of aqueous KI-I solution, adding a narrow cover slip and counting successive strips across the entire preparation. Ringing of the slip with mineral oil prevented shifting due to evaporation during the counting. The numbers of normal, partly-filled, and empty or practically empty grains were recorded. Pollen sterility showed considerable variation between anthers and between plants within the same cross, in spite of precautions taken to count the entire contents on the slide prepared from the middle third of the anther. A reasonably accurate measure of pollen sterility for a plant was considered to be furnished by separate counts of one anther each from two different spikelets on two different tassel branches, making a total of four counts.

Acetocarmine smears were used for the cytological studies, a few modifications being used for the smears of spore quartets. The proper stage was essential; if too old the spores in the quartet separated when the cover slip was added. Size of the drop of acetocarmine was important to obtain the proper degree of flattening when the cover slip was applied. To get the darker staining necessary to show the nucleolus material clearly, iron from a rusty needle (more than for pachytene smears) was worked into the drop by additional stirring. In the later work, the spores were first stained darkly, then before adding the cover slip a drop of 45 percent acetic acid was mixed in to destain the cytoplasm. This is a modification of a technique used by DR. A. E. LONGLEY for pachytene preparations in which he adds glacial acetic acid to the edge of the drop before applying the cover slip. This results in better destaining of the cytoplasm and prevents early deterioration by crystallizing of the acetocarmine. After applying the cover slip, slight heating was necessary at times to destain the cytoplasm properly. Frequency counts were made of all the different recognizable quartets on slides made in all cases from a single anther. In the later work the separated spores on the same slide were also scored as having "diffuse" or "non-diffuse" nucleolar material.

EXPERIMENTAL RESULTS FOR TRANSLOCATIONS HAVING ONE BREAK IN THE SHORT ARM OF CHROMOSOME 6 BETWEEN THE CENTROMERE AND THE NUCLEOLAR ORGANIZER (GROUP 1)

Included in this group are *T5-6c* and *T4-6* Li with one long interstitial segment; and *T2-6a*, *T6-10b* and *T5-6c* homozygous for an inversion in chromosome 5 (*In5a*), all of which have short interstitial segments.

The most extensive studies were on *T5-6c* with and without *In5a* used to shift one centromere without changing the lengths of the two axes or the

four arms of the "cross" (preliminary reports, BURNHAM 1945, 1948, 1949). I am indebted to DR. BARBARA MCCLINTOCK for suggesting the problem and furnishing seed of *T5-6c*, the inversion in chromosome 5, and a stock combining them, together with spore quartet data on *T5-6c* and *T6-10b*. The conclusion from those data that chromosomes that cross over pass to opposite poles has been reported (MCCLINTOCK 1945). The data in this paper are entirely from later material grown in the field at UNIVERSITY FARM, St. Paul, Minnesota.

Pachytene morphology

To interpret the experimental data on chromosome segregation in the crosses it is essential to know where the breakages occurred to produce the chromosomal changes. In plants heterozygous for *T5-6c*, considerable variation in the position of the "cross" was noted at pachytene. Of 21 figures observed on one

FIGURE 3. Pachytene diagram of *T5-6c*/+ with the break in 6 in the short arm adjacent to the centromere and that in 5 near the end of the long arm, 6^5 and 5^6 being the two translocated chromosomes, a the interstitial segment. The arrows show the points of breakage in inversion 5*a*.

slide, ten had the "cross" in the short arm of chromosome 6 as illustrated in the diagram (fig. 3), while eleven had it in the long arm of chromosome 6, many of them at a considerable distance. Only in the former were the homologous chromosome 6 centromeres synapsed. In the latter they were on different arms of the cross. These observations indicate the breaks were in the short arm of chromosome 6 and toward the end of the long arm of 5. A study of the two translocated chromosomes in the stock homozygous for *T5-6c* served to determine the precise breakage points, since the short arm of the normal chromosome 6 has large, darkly-staining chromomeres while the segment of the long arm of chromosome 5 distal to the knob has very small, lightly-staining ones. Since the entire short arm of the 6^5 translocated chromosome (read as "6 with a piece of 5") had only small chromomeres, the break in chromosome 6 must have been in the short arm adjacent to the centromere. The other 5^6 translocated chromosome was attached to the nucleolus, and the point where the darkly staining segment of chromosome 6 met the lightly staining one must have been the point of breakage in chromosome 5. It was considerably distal to the knob in the long arm of chromosome 5.

The breakage points in the normal chromosomes and the resulting translocated ones are shown in the diagrams, figures 4 c, d, and a, b, respectively, the average lengths of the segments in mm as measured in the *camera lucida* drawings at 5500×magnification being included. Based on these measurements, the lengths of the interchanged pieces are probably about 100 percent of the normal short arm of chromosome 6 and 11 percent of the normal long arm of chromosome 5. These observations agree with MCCLINTOCK'S state-

ment in a private communication that the breakage points were readily observable.

Pachytene stages from the stock homozygous for an inversion in chromosome 5 (*In 5a*) revealed a morphology which permitted a fairly accurate determination of the positions of these two breaks. In the inverted chromosome, a heavily stained region in the short arm adjacent to the centromere was observed in many of the figures, while in all the figures examined there was a very small knob in the long arm. This morphology would be expected if one break in the normal chromosome 5 had been in the knob present in the long arm and the other break in the short arm adjacent to the centromere. This would leave one piece of the knob adjacent to the centromere in its new position and transpose the other piece of the knob to the old position of the centromere. It is also possible that the dark region next to the centromere may have been the dark chromomeres found at that point in certain stocks

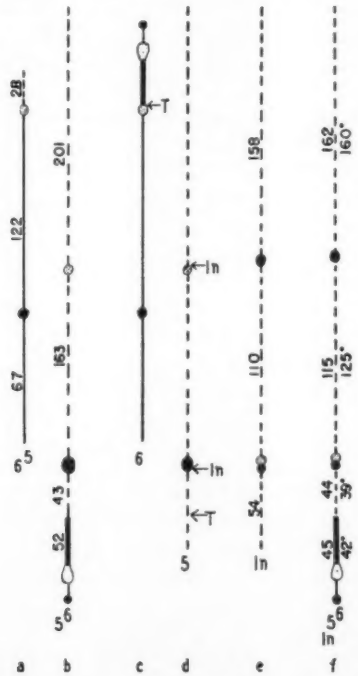


FIGURE 4. Diagrams of chromosomes 5 and 6 in homozygous normal, translocation and inversion stocks. The figures along the chromosome segments are the average lengths in mm from camera lucida drawings at about 5500×magnification. Measurements marked • on the inverted 5^6 chromosome were calculated from the separate *T5–6c* and *In5a* measurements.

a, b = the 6^5 and 5^6 chromosomes in *T5–6c*.

c, d = normal chromosomes 6 and 5, arrows indicating the points of breakage

e = chromosome 5 of *In5a*

f = inverted chromosome 5^6 of *T5–6cIn5a*.

and not a piece of the subterminal knob. In that case one break adjacent to the knob but on the distal side, and the other a short distance from the centromere in the short arm, would result in a very similar morphology. Based on the assumption that one break was in the knob, about 67 per cent of the normal long arm plus the centromere and none of the short arm were included in the inversion (fig. 4e), the points at which breakage occurred to produce the inversion being indicated on the normal chromosome (fig. 4d; only slightly different if the break had been adjacent to the knob).

The homozygous stock which has *T5-6c* and the inversion combined showed all the features of chromosome morphology at pachytene expected from what was found in the separate stocks. The 6^5 chromosome was the same as in *T5-6c*; while the 5^6 was attached to the nucleolus, had its centromere much closer to this end due to the inversion, and showed the small knob in the long arm (fig. 4f). The darkly-stained region in the short arm adjacent to the centromere was observed only in part of the figures, similar to what was described above for *In5a*. The observed average lengths of these regions (based on three cells), together with those calculated from the observed values in the separate *T5-6c* and inversion stocks (marked with ·), are also shown in figure 4f. The agreement is very close in spite of the fact that the two are based on different slides from different plants.

A study of pachytene pairing was attempted for plants heterozygous for *T5-6c* and homozygous for the inversion to determine if the shift in centromere position affected the frequency or extent of non-homologous pairing. As to the "cross" arm lengths, the configuration is expected to be similar to that shown in the diagram (fig. 3) for *T5-6c*/+ alone. Several figures were observed in which the "cross" was in the long arm of chromosome 6 indicating non-homologous pairing. More observations must be made for an adequate comparison of frequencies between this and the stock not carrying the inversion.

In the *T2-6a* heterozygote at pachytene, the "cross" was frequently far out in the long arm of chromosome 6 (BURNHAM 1932a), the chromosome 6 centromeres then being on different arms indicating non-homologous association. In an occasional figure the "cross" was at a position in which these centromeres were paired, indicating the break in chromosome 6 was at or near the centromere. Since pollen abortion in the *T2-6a* heterozygote was 50 percent, it is probable that at least part of the short arm of 6 was translocated. Examination of the homozygous stock showed the break to be in the short arm of chromosome 6, the figures not being good enough to determine the precise point. Chromosome 2^6 now has the organizer and satellite. The break in chromosome 2 was between the knob and the centromere at about L-0.4, assuming the break in 6 to be close to the centromere.

The breakage points in the translocations used may be summarized as follows:

T2-6a	2L-0.4 (or a little less)	6S-0.0 (or up to 0.5?)
T4-6Li (C. H. LI, unpublished)	4S-0.7	6S-0.2
T6-10b (MCCLINTOCK, unpublished)	6S-0.5	10L-0.58
T5-6c	5L-0.89	6S-0.0
T5-6c In 5a	(see below)	6S-0.0

In this last stock, the break in 5 is at L-0.89 in terms of the normal 5, but the centromere is now at about L-0.67; so that the distance between the new centromere position and the break point is 0.22 (0.89−0.67).

One other translocation, Connecticut 1-6, has been reported (ROBERTS 1942) to have the break in chromosome 6 in the short arm at 0.2. In the stock used for this study, only 26 percent of pollen abortion and 0.6 percent of quartets having two spores with diffuse nucleoli were observed. Examination of the homozygous stock showed the break in chromosome 6 was in the outer portion of the organizer, and both translocated chromosomes were attached to the nucleolus. The break in chromosome 1 was at about 0.05 of the long arm. Chromosome 1^6 therefore has a satellite and a small piece of the organizer region of chromosome 6 while 6^1 has retained the major portion of the nucleolar organizer region. This also accounts for the low pollen abortion since the class of spores deficient for the translocated piece of 6 probably does not abort. This translocation therefore belongs in group 3. ROBERTS has furnished his original cytological observations, including spore quartet counts. The latter showed 36 percent of "two diffuse" quartets and 4.6 percent of crossover type quartets; clearly indicating the stock I used is a different translocation.

Data on spore quartets from plants with 10 pairs of chromosomes as normal checks

For *In 5a*, based on 3,446 quartets, 0.5 percent of "two diffuse," and 0.6 percent of "one diffuse" types were observed accounting for about 0.8 percent of pollen abortion (5.4 percent observed). For *T5-6c*, based on 1,401 spore quartets, 1.1 percent of "two diffuse" and 1.8 percent with "one diffuse" were observed accounting for about two percent of pollen abortion (3.9 percent observed). One slide had six percent of abnormal quartets. In *T5-6c* homozygous for *In 5a* only 0.003 percent of "two diffuse" and "one diffuse" were observed. No data were obtained on pollen sterility on these latter plants. These cytological results may be explained on the basis of MCCLINTOCK'S conclusion (1941) that a chiasma in the short arm between the centromere and the nucleolus organizer region may result in breakage of the chromatids followed by fusion and sometimes bridge plus fragment formation. The lower observed frequency of abnormal quartets in *T5-6c In 5a* as compared with *T5-6c* is in line with this explanation since the region in which this might occur is much longer in *T5-6c*. Loss of fragments carrying the nucleolar organizers could account for the observed low frequency of spores with two nucleoli in the "one" or "two diffuse" quartets (in 35 "two diffuse" quartets, none had a second spore with two nucleoli; while in 45 "one diffuse" type, only four had a spore with two nucleoli).

A few quartets were observed in these plants in which the two "diffuse" spores were in diagonal position. These might be the result of breakage of a second division bridge in each cell of the diad. This is improbable since this would have to result from a double bridge formed by a 4-strand double in the short arm of chromosome 6. This same "diagonal type" was observed occasionally in the *T5-6c* heterozygotes. In a total of 4,054 quartets containing two

spores with diffuse nucleoli, there were 50 such quartets. As shown in figure 1B, this "diagonal type" is expected if single crossovers occur simultaneously in the two interstitial segments. This appears to be ruled out here since the pachytene morphology indicates there is only one interstitial segment. Another possibility would be occasional equational separation of both homologous centromeres at division I. A more probable explanation is the same unknown mechanism which produces it in normal plants, mentioned above.

General observations on spore quartets, abnormal types, disjunction of chromosomes which cross over

Considerable difficulty was experienced in distinguishing with certainty in all quartets the first and second division planes. Separate records were taken at the beginning of the study of those in which the two planes could be clearly distinguished; but in the later work they were not separated, since it was not necessary for the main points being studied. In certain slides, the spores in many of the quartets separate either when the cover slip is added or as heat is applied. Since the ones that do not separate might conceivably be delayed in development due to chromosomal deficiency, a comparison was made between the percentage of spores with diffuse nucleoli in the separated or single spores and that in the spores remaining in quartets. On the average there was no significant difference. Of 17 comparisons, 6 showed a difference of 8 percent or higher, differences being in both directions. Although no difficulty from this source was indicated on the average, slides showing a high percentage of spore separation were in general not used. In the later work, the separated spores as well as the quartets were scored to furnish an added basis for selection of the slides.

A few unexpected spore and quartet types were observed. Occasional spores expected to have a maximum of two nucleoli were found to have three or four. In certain plants, a few quartets were observed in which three spores or all four in the quartet clearly had the typical diffuse nucleoli. In one plant there was a high but variable frequency of quartets of this type in different anthers, occurring in sectors on certain branches. The three anthers of a given flower in these sectors were affected to varying degrees some being entirely normal. Very early prophase observations on the younger anthers in the same flowers offered no clues as to the explanation. Progeny obtained by crossing it with a normal plant showed only normal behavior. In certain plants in another stock, one or more spores of occasional quartets showed a much reduced amount of densely-stained material, this being condensed into a few irregular angular bodies not typically nucleolar. No explanation can be offered for this type of spore. They were recorded separately as abnormal quartets, but constituted a low percent.

In many of the spores expected to have two nucleoli (two spores in the "two diffuse" and one in the "one diffuse" class), only one nucleolus was observed. There was a wide variation in the frequency of this fusion. It was not a constant for a given plant or for different plants heterozygous for the same translo-

cation. However, within the same slide from a single anther there was a high correlation (r=0.80) between the probability of fusion in the "one diffuse" and the "two diffuse" classes.

If chromosomes which cross over pass to the same pole, i.e. if adjacent 2 segregation may occur following such crossovers (three-strand doubles as well as singles), "crossover type" quartets should be observed in which the two abnormal spores are on the same side of the first division plane. It was found in none of the 381 observed "crossover type" quartets in which the first division plane could be distinguished and in which the two nucleoli in the second abnormal spore had not fused. Of these, 309 were from *T5-6c*/+, as were part of the data on which McCLINTOCK (1945) based her conclusions. In this heterozygote, single and 3-strand double crossovers are estimated later to be at least 43 and 20 percent respectively. "Crossover type" quartets which have one spore with two nucleoli but in which the division plane was not recorded furnish additional evidence. Adjacent 2 segregation following crossing over would lead to the two visibly abnormal spores in the crossover type quartet being on the same side of the first division plane, while alternate and adjacent 1 segregations result in their always being on opposite sides of this plane with contiguous and diagonal positions theoretically equally frequent. If adjacent 2 segregations are frequent following crossing over, the class with the two abnormal spores in contiguous position should therefore be in excess. For *T5-6c*/+, three groups of data taken by three different people showed a consistent excess of "diagonal," the total being 2,122 diagonal and 1,929 contiguous, a highly significant deviation from 1:1, dev./S.E. being 4.3. The same was true for *T5-6c*+/+*In5a*, 1,321:1,143, dev./S.E. being 3.6; but not for *T5-6c In5a*/+*In5a*, 303:319. The fact that the diagonal class was not in excess may indicate that adjacent 2 segregation was not frequent following either single or 3-strand doubles in the interstitial segment. However, the excess of the "diagonal" class recalls the orientation of crossover chromatids in paracentric inversions (BEADLE and STURTEVANT 1935) and the excess of asymmetrical post reduction in ascomycetes reported by HALDANE and WHITEHOUSE (1944). As they suggest, such a result might be expected if crossing over tends to produce such an orientation of the crossover chromatids which persists through interphase. If the crossover chromatids are oriented more often toward the middle non-functional positions in the linear quartet of megaspores in species of higher plants, a lower frequency of recovered crossovers in female than in male gametes might be expected.

3-1 segregation of chromosomes from the ring

Since the frequency of 3-1 segregation in the ring could account for some of the observed pollen sterility (due to abortion of n−1 spores) chromosome counts were made at meiosis. In plants heterozygous for *T5-6c*, counts on 65 late prophase II diads in which chromosome number could be determined in both cells showed only one which probably was a 9-11 segregation. The chromosome number was counted in 268 separate cells at late prophase II, one having 9 chromosomes; one a doubtful 9 and one an uncertain 11. These may

not represent 3-1 segregation in the ring, but even if they do the rate was very low.

In plants homozygous for the inversion and heterozygous for *T5-6c*, 38 separated cells were counted at late prophase II, all having 10 chromosomes. The numbers are inadequate but again indicate the deviation from normal segregation is not high at the first division. In neither stock does it appear to account for any appreciable amount of pollen abortion.

Chromosome segregation, spore quartet data

Group 1—Translocations with one break in the short arm of chromosome 6

a. *with short interstitial segments* including *T5-6c In5a*, *T2-6a* and *T6-10b*. A summary of the data for these has been presented (BURNHAM 1949), but are presented here in greater detail. Plants heterozygous for *T5-6c* and homozygous for *In5a* were produced by crossing a stock homozygous for both *T5-6c* and *In5a* with one homozygous for *In5a*. Figure 3 represents the pachytene configuration, except that both chromosome 5 centromeres are shifted to the point near the knob indicated by the left arrow. Figure 4, e and f represent the two number 5 chromosomes. The detailed data are shown here in table 1, together with the calculated frequencies of the segregation types in the non-crossover quartets. The three groups of plants for which sub-totals are given in the table were from different crosses of related material. There was considerable variation between crosses and between plants from the same cross. With one exception, the plants in culture 6,913 showed much lower percentages of quartets having one or two "diffuse" spores than did the others. The reason for this difference is not known. One plant, 6,913–7, had only 41 percent of pollen abortion and consequently a very low calculated frequency of adjacent 2 segregation. It was not a 2n+1 plant. As shown earlier, a low percentage of the abnormal quartets, less than two percent, was observed in the homozygous controls, but since the predicted pollen abortion probably includes most of these (and in general probably more than indicated by the quartet phenotype) no correction was used. Even if the observed abortion in the translocation heterozygotes were corrected for the full amount, the conclusions would be essentially the same since this would reduce the frequency of adjacent 2 segregation by only two to five percent. The observed frequency of "crossover type" quartets, 10.6 percent for the grand total in table 1, is so low that probably no double crossing over occurred. Hence this is the percentage of single crossing over in the interstitial segment of chromosome 5, and, as shown in figure 1B, may be the result of alternate or adjacent 1 segregations following these single crossovers. Since there were probably no double crossovers, the "two diffuse" type is the result of adjacent 1 segregation in chromatid tetrads which had no crossing over in the interstitial segment. There were 27.8 percent of this type. Since the "crossover type" quartet should produce two and the "two diffuse" type should produce four aborted spores, the predicted pollen abortion for the total of all the data was 33.0 percent. The observed pollen abortion was 50.5 (table 1), an excess of 17.5 percent which should measure the frequency of adjacent 2 segregation (normal-ap-

TABLE 1

Summary of spore quartet and pollen abortion data, including frequencies of segregation types, for a group 1 translocation heterozygote (one break in the short arm of chromosome 6): T5-6c homozygous for the inversion in chromosome 5 (In5a); a short interstitial segment.

CULTURE PLANT NO.	NO. OF SLIDES	SPORE QUARTETS					POLLEN				NON-CROSSOVER QUARTETS			
		NO DIFFUSE	TWO DIFFUSE	ONE DIFFUSE*	TOTAL	PERCENT CROSS-OVER TYPE	TOTAL	ABORTION, PERCENT			TOTAL	SEGREGATION TYPES IN %		
								OBSERVED	PREDICTED	PARTLY† FILLED		ALTERNATE	ADJACENT 1	ADJACENT 2
6913- 3	2	442	145	51	638	8.0	2,486	51.1	26.7	9.5	587	48.7	24.7	26.6
-29	2	694	235	48	977	4.9	2,958	53.1	26.5	15.7	929	46.7	25.3	28.0
- 7	1	210	111	64	385	16.6	3,008	41.0	37.1	11.0	321	60.7	34.6	4.7
-12	2	602	239	79	920	8.6	3,370	49.8	30.3	11.7	841	50.3	28.4	21.3
-14	2	469	137	51	657	7.8	2,223	47.1	24.7	12.5	606	53.1	22.6	24.3
-18	3	436	150	44	630	7.0	1,907	50.4	27.3	12.8	586	49.5	25.6	24.9
subtotal		2,853	1,017	337	4,207	8.0	15,952	48.7	28.2	12.2	3,870	51.4	26.3	22.3
6924- 1	2	565	314	116	995	11.7	2,074	55.8	37.4	17.3	879	43.5	35.7	20.8
-10	2	288	186	133	607	21.9	1,874	53.9	41.6	14.0	474	44.9	39.2	15.8
subtotal		853	500	249	1,602	15.5	3,948	54.9	39.0	15.7	1,353	44.2	37.0	18.8
6925- 9	2	435	282	83	800	10.4	2,672	51.6	40.4	—	717	48.1	39.3	12.6
6926- 8	2	293	197	90	580	15.5	3,187	51.1	41.7	—	490	48.6	40.2	11.2
subtotal		728	479	173	1,380	12.5	5,859	51.3	41.0	—	1,207	48.5	39.7	11.8
Total		4,434	1,996	759	7,189	10.6	25,759	50.2	33.0	12.9	6,430	49.7	31.0	19.2

* Crossover type.
† Observed.

pearing quartets but all aborted spores). Of the normal-appearing quartets 1,258 (17.5 percent of 7,189, the total number of quartets) were from adjacent 2 segregation; and the remainder were from alternate segregation. Of the non-crossover quartets, the percentages resulting from alternate, adjacent 1, and adjacent 2 segregations were therefore 49.4, 31.0 and 19.6 respectively. If alternate and adjacent 1 segregations are assumed to be equally frequent following crossing over in the interstitial segment, these values when the crossover quartets are included become 49.5, 33.0 and 17.5 percent, respectively; a small change in the ratio. The percentage of crossover quartets varied from 4.9 to 21.9 percent while the percentage of adjacent 2 segregations varied from 4.7 to 28.0. The correlation between the two is -0.77 which, for 10 comparisons, is highly significant ($P=0.01$). In other words, the higher the frequency of crossing over, the lower the frequency of adjacent 2 segregation. In the non-crossover quartets, alternate segregations accounted for about 50 percent while adjacent 1 and 2 together accounted for the other 50 percent. This may be accounted for if for each "open" ring a "zigzag" one is equally likely. The average ovule abortion, 49.3 percent, did not differ significantly from the observed pollen abortion, 50.5.

Translocation 2–6a. The spore quartet and pollen data from plants heterozygous for this translocation are in table 2. Only one percent of crossovers in the interstitial segment (probably mainly in chromosome 2) was observed. Although this segment in chromosome 2 was considerably longer than in *T5-6c In5a* (as long as 40 percent of the long arm, not less than 30 percent, depending on whether the break was near the centromere or near the organizer of chromosome 6), crossing over was much lower. The difference between observed and predicted pollen abortion, 25.9 percent, measures the frequency of adjacent 2 segregation. Based on the non crossover quartets, there were 25.6 percent of adjacent 2, 26.0 percent of adjacent 1 and 48.4 percent of alternate segregation.

Translocation T6-10b. In plants heterozygous for *T6-10b* (breaks at 6S-0.5 and 10L-0.58, McCLINTOCK unpublished), the data in table 2 also show the occurrence of both adjacent 1 and 2 segregations, the values being 23.4 and 19.1 percent, respectively. The ratio of alternate:total adjacent was 58.0:42.1. The excess of alternates is significant, and is reflected in a higher percentage (57.1) of normal pollen. This may represent an actual excess of alternate segregation, although it is possible that the deficiency for 0.5 of the short arm of chromosome 6 does not always cause complete abortion. In that case alternate segregation may not be much in excess of 50 percent. The percentage of crossover quartets, 5.3, was considerably lower than for the heterozygous *T5-6c* stock homozygous for inversion 5a although the relative length of the chromosome 10 interstitial segment in *T6-10b* is roughly twice that in the other. Translocation *6-10b* may bring about a greater reduction in crossing over, or the amount of crossing over per unit length in this region of this chromosome may normally be much lower. In these translocations, the interstitial segment in chromosome 6 where present as in *T6-10b* and possibly *T2-6a* is so short in relation to the other interstitial segment that little of the observed crossing over probably occurred in it.

TABLE 2

Summary of spore quartet and pollen abortion data including frequencies of segregation types for other group 1 translocations (one break in the short arm of chromosome 6); short interstitial segments.

TRANSLOCATION HETEROZYGOTES	SPORE QUARTETS					POLLEN			NON-CROSSOVER QUARTETS			
	NO DIFFUSE	TWO DIFFUSE	ONE DIFFUSE*	TOTAL	PERCENT CROSS-OVER TYPE	TOTAL	ABORTION PERCENT		TOTAL	SEGREGATION TYPES IN PERCENT		
							OBSERVED	PREDICTED		ALTERNATE	ADJACENT 1	ADJACENT 2
2-6a(10441-1)	972	348	20	1,340	1.5	2,473	49.9	26.7	1,320	50.1	26.4	23.6
2-6a(10442-2)	766	250	5	1,021	0.5	2,478	53.3	24.7	1,016	46.7	24.6	28.7
Total	1,738	598	25	2,361	1.0	4,951	51.6	25.9	2,336	48.4	25.6	26.0
6-10b(10475-2)	485	129	33	647	5.1	9,820	44.9	22.5	614	55.4	21.0	23.6
6-10b(10425-3)	1,334	300	93	1,727	5.4	6,992	40.0	20.1	1,634	60.6	18.4	21.0
Total	1,819	429	126	2,374	5.3	16,812	42.9	20.7	2,248	57.5	19.1	23.4

* Crossover type.

The data thus far presented are in agreement in showing that when the interstitial segment is short in terms of a low frequency of crossing over, i.e. a high frequency of chromatid tetrads with no crossovers in this segment, both types of adjacent segregation occur even though one axis is extremely short as in the two *T5-6c*/+ stocks where the ratio of the two axes is about 1:7.45. Since the evidence indicates that chromosomes which cross over pass to opposite poles, the adjacent 2 segregations occurred in the meiocytes having no crossing over in the interstitial segment.

The negative correlation between the frequency of crossing over and that of adjacent 2 segregation which was shown earlier for the individual plants in *T5-6c*/+ homozygous *In5a* is also shown in the following tabulation from tables 1 and 2:

	T5-6c In /+*In*	*T6-10b*	*T2-6a*
Percent C.O.	10.6	5.3	1.0
Percent adjacent 2 (based on total quartets)	17.5	22.0	25.8

The differences are not great but are in agreement with this relation.

Group 1b. Translocations with at least one long interstitial segment; T5-6c, T4-6 Li. Similar data on quartets and pollen together with the calculated segregation frequencies for plants heterozygous for *T5-6c* without the inversion and for *T4-6* Li are in table 3. For *T5-6c*, figure 3 represents the pachytene configuration, fig. 4*a*, *b* the translocated chromosomes. Based on 4,229 quartets from six plants (three crosses) an average of 62.8 percent were of the "crossover type," the value ranging from 46.4 to 70.6 percent for individual plants. There were 742 quartets or 17.5 percent of the "two diffuse" type. Since the observed pollen abortion (48.1 percent) was no greater than the predicted (49.3), actually a little lower, no measurable amount of adjacent 2 segregation is indicated.

The degree of ovule abortion was 47.6 percent, practically the same as the observed pollen abortion, 48.1, indicating little if any megaspore competition. BRIEGER (1945) has presented what he considers as evidence for such competition in corn.

For *T4-6* Li, the average percent of crossover type quartets was 62.6 percent, and again the predicted abortion was close to the observed 50 percent indicating very little adjacent 2 segregation. The high percentage (63) of "crossover type" quartets for both translocations indicates that multiple crossovers were frequent in the interstitial segment. The frequency of adjacent 1 segregations cannot be calculated directly, since the "two diffuse" quartet is expected following 2-strand and 4-strand doubles as well as from non crossovers (see fig. 1B). For further analysis, an estimate of the frequency of chromatid tetrads with 0, 1 and 2 points of crossing over in this segment is desirable but will be attempted only for *T5-6c*. The genetic length of this segment corresponds roughly to the *bm-pr-v2* region, since *bm* is close to the centromere and *v2* is between the knob and the end of the long arm (the latter based on unpublished *T5-9a* data). In normal material, recombination in the *bm-pr* region is about 23 percent and in *pr-v2* about 44 percent with about 8 percent

TABLE 3

Summary of spore quartet and pollen abortion data, including frequencies of segregation types, for two group 1 translocation heterozygotes (one break in the short arm of chromosome 6): T5-6c and T4-6Li; one very long interstitial segment.

CULTURE, PLANT NO.	SPORE QUARTET							POLLEN				SEGREGATION TYPE
	NO. SLIDES	NO. DIFFUSE	TWO DIFFUSE	ONE DIFFUSE	TOTAL	PERCENT "TWO" DIFFUSE	PERCENT CROSS-OVER TYPE	TOTAL	ABORTION			PERCENT ADJACENT 2
									OB-SERVED	PRE-DICTED	PARTLY FILLED	
T5-6c/+												
6899- 1	2	175	155	286	616	25.2	46.4	1,835	50.0	48.4	24.3	1.6
- 4	5	104	129	375	608	21.2	61.7	5,637	42.0	52.1	20.4	0.0
6900- 6	3	178	157	674	1,009	15.6	66.8	2,318	51.4	49.0	24.2	2.4
6901- 7	2	124	108	406	638	16.9	63.6	2,163	48.5	48.7	22.4	0.0
- 9	3	97	102	477	676	15.1	70.6	2,065	48.7	50.4	21.8	0.0
-10	2	131	91	460	682	13.3	67.4	2,733	47.7	47.1	20.3	0.6
Total		809	742	2,678	4,229	17.9*	62.8*	16,751	48.1*	49.3*	22.2*	0.0
T4-6Li												
15085- 3	2	163	109	319	591	18.4	54.0	2,883	48.0	45.4	15.3	2.6
15086- 1	2	83	70	378	531	13.2	71.2	1,991	51.6	48.8	—	2.8
Total		246	179	697	1,122	15.8*	62.6*	4,874	49.8*	47.1*	—	2.7

of doubles (EMERSON et al. 1935). A higher value (34 percent) in male flowers has been reported for *bm-pr* (RHOADES 1941). A linkage test with semisterility, based on small numbers, showed 30 percent recombination with *pr*. Since both regions are long, a total of 10 percent of recovered doubles from the pollen seems to be a conservative estimate, even with a considerable reduction in crossing over in the region adjacent to the break. No estimate of the higher multiples is attempted. Using SAX's (1932) and WEINSTEIN's (1929, 1936) method of calculating chiasma frequency assuming randomness as to the non sister chromatids involved, to obtain 10 per cent of recovered doubles, four times as many sporocytes, or 40 percent, must have had two crossover regions in the interstitial segment, of which there should have been 10 percent of 2-strand, 20 percent of 3-strand and 10 percent of 4-strand doubles. It was shown earlier that the chromosomes involved in 3-strand doubles as well as in singles pass to opposite poles. What type of segregation follows the 2- and 4-strand doubles is not known. However, if adjacent 2 segregation occurred as frequently following these crossovers as following no crossing over (roughly 20 percent), as much as four percent of adjacent 2 might have been expected which should have been reflected in higher pollen abortion than that predicted. Since this calculation is based on an estimate of the frequency of doubles which is more likely to be low than high, it is probable that for all double as well as single crossovers the chromosomes which cross over pass to opposite poles. This leaves alternate and adjacent 1 as the two possible segregations following these crossovers. A higher frequency of alternates would, if 2-strand and 4-strand doubles were equally frequent, result in an excess of "no diffuse" over "2-diffuse" quartets (since with alternate segregation non crossover tetrads as well as 2-strand doubles result in "no diffuse" whereas only 4-strand doubles result in "two diffuse" quartets, fig. 1B). For *T5-6c*/+ 809 "no diffuse": 742 "two diffuse" were observed (table 3), the excess of "no diffuse" not being significant. Although the slight excess is in the direction indicating more frequent alternate segregation, it could result also from a higher frequency of 2-strand doubles with an equal frequency of alternate and adjacent segregation. Since the data for the group 1 translocations reported here indicate little or no directed segregation in the non crossover tetrads, equal frequencies of the two segregations are assumed following crossing over in the interstitial segment. With these assumptions, the observed data in table 3 (using the totals) may be used for the following calculations:

	NO DIFFUSE	TWO DIFFUSE	ONE DIFFUSE
2-strand doubles, 10 percent of 4,229	212	212	—
3-strand doubles, 20 percent of 4,229	—	—	848
4-strand doubles, 10 percent of 4,229	212	212	—
Total	424	424	848
Observed (total of 4,229 quartets):	809	742	2,678
Difference:	385	318	1,830

The difference between the number of observed "one diffuse" quartets and the calculated number resulting from 3-strand doubles, (2,678−848 or 1,830, i.e. 43 percent) would be the tetrads with one crossover. The differences between observed and calculated numbers in the "no diffuse" and "two diffuse" classes, 385 plus 318 or 17 percent, would be the no-chiasma tetrads. Thus, the percentages of tetrads with 2, 1 and 0 crossovers are estimated to be: 40, 43 and 17 respectively, or 83 percent for which adjacent 2 segregation would not be expected. If 20 percent of the remaining 17 had adjacent 2 segregation,

TABLE 4

Counts of frequencies of different types of translocation configurations at diakinesis, in T5-6c/+ without the inversion and with it homozygous.

	RING	COLLAPSED FIGURE-OF EIGHT	CHAIN	TWO PAIRS	TOTAL	DOUBTFUL
T5-6c/+						
6899-1,-4,-15	419	26	171	12	628	34
percent	66.7	4.1	27.2	1.9		
6900-3,-6	344	109	236	13	702	120
percent	49.0	15.5	33.6	1.9		
Grand total	763	135	407	25	1330	154
percent	57.4	10.2	30.6	1.9		
T5-6c Inv. 5 / +Inv. 5						
6913-4,-7,-12	254	81	233	7	575	100
percent	44.2	14.1	40.5	1.2		
6925-9, 6926-8	150	49	234	7	440	32
percent	34.1	11.1	53.2	1.6		
grand total	404	130	467	14	1015	132
percent	39.8	12.8	46.0	1.4		

as in the translocations with a short interstitial segment, about three percent of adjacent 2 might have been expected. If doubles were higher, as seems probable, this would be lower.

The evidence from these two translocations indicates that when most of the tetrads have a crossover in the interstitial segment, there is little or no adjacent 2 segregation.

Partially-filled pollen. The differences in segregation between the two *T5-6c/+* stocks (with and without the inversion) are also reflected in the frequencies of partially-filled pollen grains, roughly 25 to 50 percent filled with starch. They were clearly distinguishable from the normal class, but a few were not as sharply different from the ones classed as empty which occasionally had scattered starch contents. The data are in column 11 of table

1 and column 12 of table 2. In the group carrying only *T5-6c*, 21.8 percent of the pollen were partially filled, while in the group carrying *T5-6c* but homozygous for the inversion only 12.9 percent were of this type. It is probable that the partially filled pollen is the class deficient only for the translocated piece of the long arm of chromosome 5, since it is very short amounting to only 11 percent of that arm (a study of the two tertiary trisomics from this translocation would check this). In the "two diffuse" quartets two spores, and in the crossover type only one should be deficient for this short piece. Based on the quartet data in tables 1 and 2, the calculated percentages are 24.6 and 13.1, respectively which agree rather closely with the observed (21.8 and 12.9).

Diakinesis observations on T5-6c/+ stocks. Data on the various configurations seen at diakinesis in the two stocks (with and without the inversion) should also have a bearing on the problem, unless there is no relation between these and the configurations the translocation complex will take as it disjoins at late metaphase I or anaphase I. Summaries of counts on the various configurations at diakinesis in the two stocks are in table 4. In the one homozygous for the inversion, there was a significantly higher frequency of chains (46 vs. 31 percent) and a lower frequency of rings (40 vs. 57 percent). In both, the chains were mainly the result of failure to remain associated in the short translocated piece of chromosome 5, i.e., the arm opposite the one attached to the nucleolus. Of 874 chains in both stocks, one clearly failed to be together at the ends attached to the nucleolus and three failed to pair in a long arm of the cross. In eight figures, the ring was broken into two "pairs" attached at opposite sides of the nucleolus. Others were undoubtedly not synapsed in the short arm of chromosome 6, but appeared to be because of their association with the nucleolus. At metaphase after the nucleolus is no longer present to bind these ends together, the actual number of "pairs" may be much higher. The frequency of chains does not seem to be closely related to the frequency of crossing over in the interstitial segment: 31 vs. 46 percent of chains respectively, whereas the corresponding percentages of "crossover type" quartets were 63 and 11. It is possible that the shift of the centromere to a position nearer the translocation point results in reduced crossing over in adjacent regions including the short arms which represent the translocated pieces, thus accounting for the higher frequency of chains. Evidence for the absence of adjacent 2 from chain configurations (in *T1-6b*, *T5-6b* and *T3-6b*) will be discussed later (tables 5 and 6). The higher frequency of chains in the *T5-6c/+* stock homozygous for the inversion means a larger number of sporocytes in which adjacent 2 segregations probably would not occur. Although the opposite was observed, an exact relationship may not be expected, since only 20 to 25 percent of the configurations not showing crossing over in the interstitial segment appear to be followed by adjacent 2 segregation (table 1).

At the later stages of meiosis, a comparison of plants heterozygous for *T5-6c* with the inversion homozygous and without the inversion showed the former to have a somewhat higher frequency of abnormalities at division I anaphases (2.6 vs. 0.3 percent), but a lower frequency at division II (1.3 vs.

6.6 percent). These abnormalities included lagging chromosomes or micronuclei at division I and lagging chromosomes at division II. No explanation for these differences is offered at present.

Assuming that the above frequencies of the different kinds of configurations in the two stocks hold for the later stages (rings might become chains later, but the reverse is not expected), the frequencies of the different kinds of segregations may be calculated. For the chains no adjacent 2 segregation is assumed. For the rings in which crossing over had occurred, a ratio of 1 alternate:1 adjacent 1 is assumed with no adjacent 2. For the rings without crossing over, the percentage of adjacent 2 used is the one observed for *T5-6c*/+ homozygous *In5a* i.e. 19.6. The crossovers are assumed to be equally divided between the rings and the chains and "pairs." The calculated results are the following (*T5-6c*/+ is omitted since no adjacent 2 segregation was observed):

		TOTAL PERCENT	ALTERNATE	ADJACENT 1	ADJACENT 2
T5-6c In5a / *+In5a*	rings and collapsed figure of eights				
	(without c.o.)	47.3	23.6	4.0	19.6
	(with c.o.)	5.3	2.7	2.7	0.0
	chains and pairs (with or without c.o.)	47.4	23.7	23.7	0.0
	Total	100.0	50.0	30.4	19.6

If alternate segregations occur in 50 percent of all tetrads and the adjacent 2 segregations occur only from rings in which there has been no crossing over in the interstitial segment, then adjacent 1 segregation from the rings in *T5-6c*/+ is very low, only 6.7 percent. This may be due to the high frequency of chains in *T5-6c*. In *T2-6a* with a higher frequency of rings, adjacent 1 segregation from the rings should be more frequent.

The differences in segregation between the two stocks of *T5-6c*/+, one homozygous *In5a*, the other lacking it, were achieved without any change in relative lengths of the two axes, the only difference being in the length of the interstitial segment. In a preliminary report (BURNHAM 1945), the differences in segregation between them were interpreted as due to a centromere effect. Its importance is now interpreted to be in determining the length of the interstitial segment and consequently the amount of crossing over there.

Group 2. Translocations with the break in 6 in the long arm or in the satellite[3]

In this group, the "two diffuse" type of quartet results from adjacent 2 segregation, whether or not crossing over has occurred in an interstitial seg-

[3] The assistance furnished by the Biology Department of the CALIFORNIA INSTITUTE OF TECHNOLOGY which made the completion of these data possible is gratefully acknowledged. MR. T. GISH aided in the spore and pollen counts.

TABLE 5

Summary of breakage positions, spore quartet and pollen abortion data for group 2 translocation heterozygotes (break in chromosome 6 in the long arm or in the satellite); both interstitial segments short. The break in the other chromosome was in the long arm unless indicated by S, as S.17 for 1-6c. Authority for the breakage positions, many unpublished: A = E. G. ANDERSON, B = BURNHAM, L = LONGLEY (1948).

TRANS-LOCATION	CHROM. 6	OTHER CHROM.	AUTHORITY	SPORES		ONE DIFFUSE QUART. (PERCENT)	ADJACENT 2 (PERCENT)	POLLEN		
				TOTAL	DIFFUSE			TOTAL	PERCENT ABORTED	PERCENT PARTLY FILLED
T1-6a	.52	.14	B	16,104	1,251	0.1	15.5	10,291	51.5	—
1-6*b*	sat.	.25	B 1932	17,356	11	0.07	0.06	58,000	26.3	—
1-6*c*	.39	S.17	L	1,053	172	0	32.6	—	—	—
2-6*c*	.20	.32	L	944	64	0	13.6	2,224	48.0	2.7
2-6*d*	.3	.3	L	1,200	200	1.3	33.3	2,261	47.6	7.5
3-6*a*	.19	.07	L	2,613	331	—	25.4	2,556	57.2	7.7
4-6*a*	.3	.2	L	4,451	577	0.2	26.0	20,984	56.0	few
5-6*a*	.3	.1	B	3,990	305	1.7	15.2	665	50.2	few
5-6*b*	sat.	.1(?)	B	10,124	46	0.3	0.9	2,843	19.9	—
5-6(5786-1)	.2	.2S? or L?	B	2,197	278	0.5	25.4	3,094	50.2	few
6-9*b*	.2	.2(?)	A 1938	12,388	1,662	0.5	26.8	2,083	50.2	3.8
6-9(A66)	.20	.17	L	1,294	233	—	36.0	—	—	—
							25.0 (average, omitting 1-6*b* and 5-6*b*)			

ment (fig. 2). All quartets and separated spores were recorded, the latter being scored as having diffuse or non-diffuse nucleolus material. An attempt was made to examine enough quartets to determine the frequency of the "one diffuse" class which might arise from certain of the possible 3-1 segregations. Table 5 summarizes the data for twelve translocations in which both interstitial segments are probably genetically short, while table 6 summarizes the data for nine in which one or both segments are long. Included are the breakage point locations and pollen abortion data as well as the spore and quartet counts. The aborted class includes the partly filled pollen grains. The percentage of adjacent 2 segregation was calculated by doubling the observed percentage frequency of diffuse-nucleolate spores (including quartets as well as separated spores), since two of the four spores from the "two diffuse" quartets would be scored as "non-diffuse." This value is in error by half the frequency of "one diffuse" quartets, but even in *T4-6b* with 3.4 percent of "one diffuse" quartets (table 6) the error is relatively small. With the exception of *T1-6b* and *T5-6b* which form only chains, and are considered below, the translocations in table 5 had relatively large amounts of adjacent 2 segregation, ranging from 14 to 36 percent, with an average of 25 percent. The large differences between them may be due to differences in frequencies of crossing over in the interstitial segments as already shown for the group 1 translocations. Unfortunately crossovers can not be determined cytologically in this group.

Samples for pollen abortion counts were not available for all the translocations, but those obtained do not deviate very widely from 50 percent of normal pollen this being the percentage of alternate segregation (except *T1-6b* and *T5-6b* which are considered below). In no case is there higher pollen fertility which might indicate directed segregation. The difference between the observed percentages of adjacent 2 segregation and of pollen abortion should represent the frequency of adjacent 1 segregation. On this basis, adjacent 1 segregations probably ranged from 36 to 14 percent for the different translocations, with an average of 25 percent.

The two exceptions in table 5 are *T1-6b* and *T5-6b* with very little adjacent 2 segregation, 0.07 and 0.3 percent respectively. In both, the break in chromosome 6 was in the satellite, the heterozygotes always showing chains at meiosis due to failure of synapsis of the satellite arm of the pachytene "cross." Making allowance for the fact that their low sterility is due to the non-abortion of one class of deficient spores, there was probably about 50 percent of adjacent 1 and 50 percent of alternate segregation in *T1-6b* and 45 and 55 percent in *T5-6b*. Cytological observations on *T1-6b*/+ at metaphase I also indicated that the only type of adjacent segregation which occurred was that in which two chromosomes, one at each end of the chain, passed together to the same pole i.e. adjacent 1 (BURNHAM 1932b). In *T1-6b* and *T5-6b*, the interstitial segments are so short, the translocated piece of 1 and of 5 respectively being long, that little crossing over is expected. In rings with short interstitial segments, an average of 25 percent of adjacent 2 segregation was observed, whereas in chains with short interstitial segments there was very little. Similar

TABLE 6

Summary of breakage point positions, spore quartet and pollen abortion data for group 2 translocation heterozygotes (break in 6 in the long arm or in the satellite); one or both interstitial segments long. The break in the other chromosome was in the long arm unless indicated by S.

TRANSLOCATION	CHROM. 6	OTHER CHROM.	AUTHORITY*	SPORES		ONE DIFFUSE QUART. (PERCENT)	ADJACENT 2 (PERCENT)	POLLEN		
				TOTAL	DIFFUSE			TOTAL	PERCENT ABORTED	PERCENT PARTLY FILLED
2-6 (6049)	.23	S.7	B	4,059	197	1.1	9.8	2,628	47.0	26.5
2-6 (6052)†	.6	S.6	B	6,590	10	0.0	0.3	3,383	48.1†	17.5
3-6*b*	sat.	S.75	C.A.	3,403	0	0.0	0.0	2,332	24.8	22.7
3-6 (Conn.)	.6	S.4	R	14,386	310	0.1	4.4	11,264	49.9	20.0
4-6*b*	.2	S.8	A 1940	3,571	139	3.4	7.8	1,934	42.0‡	17.4
5-6 (Bikini 3063)	.9?	.9?	L	4,625	14	0.0	0.6	1,128	49.5	41.2
6-8*a*	.5	.7	A 1939a	3,822	334	0.0	17.4	2,958	48.0	—
6-8 (D1)	.5	.8	L	1,087	6		1.1	—	—	—
6-10*a*	.7	.1	A., R.	1,319	10	0.0	1.5	2,066	53.9	15.5
						Ave.	3.2 (omitting *T6-8a*)			

* A = E. G. Anderson, B = Burnham, L = Longley 1948, R = Roberts 1942, C.A. = Clarke and Anderson, 1934, A., R. = Anderson and Randolph 1945.
† Some plants high, above 60 per cent pollen abortion.
‡ Probably not all the slightly smaller and nearly filled but abortive grains were included.

results have been reported by McCLINTOCK (1934) for *T6-9a* (breaks in the nucleolar organizer of 6, and 0.3 of the long arm of 9) with more than 50 percent of chains and a short interstitial segment. By cytologically identifying the chromosomes in the microspores, she determined that adjacent 2 segregation was infrequent and that one of the two classes of spores from adjacent 1 segregation occurred in 25 percent of the spores, i.e. a total of about 50 percent of adjacent 1. Less than ten percent of adjacent 2 segregations might have been expected based on the frequency of rings. However, rings were the result of association in the arm of the "cross" consisting of part of the nucleolar organizer plus the satellite, and may not have been comparable to the association which normally makes up an arm of a ring. Chains, at least those involving chromosome 6, appear to form a special group in which adjacent 2 segregation is rare in spite of the presence of short interstitial segments.

Table 6 includes nine translocations in which either or both interstitial segments are probably long enough to permit a high frequency of crossing over. With one exception the frequency of adjacent 2 segregations was low, the average of all being 4.8 percent. The value for *T2-6* (6049) was also high but the 17.4 percent value for *T6-8a* was the only one which overlapped those in table 5. The difference between *T6-8a* and *T6-8(D-1)* with 1.1 percent cannot be explained since the breaks were in about the same positions. Quartet counts on another culture of *T6-8a*/+ gave 8 percent for one plant and 12 for another. Further studies are in progress to determine if some extraneous factor is present in certain plants.

Since in the translocations listed in table 6 either or both translocated pieces were relatively short (as evidenced by higher percentages of partly filled pollen), higher frequencies of chains or of "pairs" are expected. Adjacent 2 segregations should not occur from the "pairs" since they result from failure of association in alternate arms, i.e. the two translocation arms of the cross. No observations were made on the frequencies of rings, chains and "pairs" for the translocations in table 6, but it is certain that many of them had a frequency of rings sufficiently high to make crossing over in the interstitial segment the factor which decreased adjacent 2 segregation.

The *3-6b* translocation with one break in the satellite (25 percent pollen abortion) furnishes evidence on the segregation from chains. For this heterozygote CLARKE and ANDERSON (1935) reported 31 percent of chains at diakinesis, the remainder being "pairs." In the later cross used for these studies, 18 percent of chains were observed, and no adjacent 2 segregation (table 6).

One other translocation, not listed in table 6, with a break in the satellite and a long interstitial segment, 5-6 (A75) with breaks at 5 S-O.54 and 6 sat. also showed no adjacent 2 segregation. A similar lack of this type of segregation was found for the chains in *T5-6c* (table 3), (30.6 percent of chains observed at diakinesis, table 4).

No estimate of the frequency of adjacent 1 segregation in the translocations in table 6 can be made, because of the unknown frequency of crossing over in the interstitial segments which give rise to "no diffuse" quartets with two

normal and two abortive spores. The data in tables 1 to 6 indicate that in rings the length of the interstitial segment is an important factor in determining the frequency of adjacent 2 segregation. Chains form a special group in which adjacent 2 segregation is rare, no matter how little or how much crossing over occurs in that segment.

In most of the translocations studied the lengths of the two axes were nearly equal or the axis carrying the two centromeres was considerably longer, for example, the centromere-carrying axis comprised more than 90 percent of the total length in *T5-6c*, *3-6b*, and *5-6* (Bikini 3063). In three, *T3-6a*, *6-9b* and *6-9* (A66), it was considerably shorter, with 34, 38 and 37 percent respectively of the total length; yet the percentages of adjacent 2 segregation, 25, 27 and 36 respectively (table 5), were similar to the others. Therefore the presence of the centromeres on the short axis does not upset the relation between interstitial segment length and adjacent 2 segregation.

Correlation between segregation and lengths of other chromosome segments

Crossing over in other segments might be expected to be important also. The correlation coefficients between the frequencies of adjacent 2 segregations and the lengths of chromosome segments, or ratios of these lengths, were calculated for 24 translocations. Only the one with the length of the longer interstitial segments, $r = -.509 \pm .183$, approached significance at $P = 0.01$; that with the total length of the interstitial segments, $r = -.375 \pm .198$, approached significance at $P = .05$; that with the total of the non-translocated arms was negative but low and not significant. Except in special stocks in which the centromere is shifted by means of an inversion (as in the two *T5-6c* stocks reported here), the total length of the interstitial segments decreases as the total length of the translocated pieces increases. Since the other ratios of lengths are affected by this relationship, only correlation values above those for interstitial segments would indicate a similar effect of the lengths of other segments. Since all the other values were lower, although a few were significant or approached $P = .05$, it appears that the frequency of crossing over in the other segments is not important in determining segregation in maize translocations, unless they have an opposite effect.

DISCUSSION

Several studies of chromosome segregation in translocations in *Drosophila melanogaster* have been made, using the cross of heterozygote×heterozygote with marker genes to permit recognition of the segregation types. Deficiency-carrying gametes unite with complementary duplication-carrying ones to produce viable zygotes. To permit comparisons, the published frequencies from those experiments have been recalculated in terms of gametic frequencies representing the different kinds of segregations. Corrections were made for differences in the number of combinations expected to survive and for overlapping phenotypes. The square roots of these figures are given as the gametic frequencies for a given segregation on the assumption that the actual fre-

quencies were the same in ♂ and ♀ (this may not be true, as will be shown later). The breakage positions and the calculated gametic frequencies are summarized below:

TRANSLOCATION	CHROMOSOMES	BREAKAGE POSITIONS		CALCULATED GAMETIC RATIO IN PERCENT		EGG COUNTS	
		FIRST CHROM.	SECOND CHROM.	ALTERNATE	ADJACENT	N×T/+(♂)	T/+×N
A (D&S)	2–3	near centromere	near centromere	61 :	19 : 21	60	
C (D&S)	2–3	near centromere	near centromere	66 :	19 : 16	60	
B (D&S)	2–3	near δ	near centromere	63 :	37 : 0	48	
E (D&S)	2–3	farther out on arm	farther out on arm	51 :	49 : 0	—	
Pipkin	2–3	near centromere	near centromere	59 :	25 : 13	57	53
V3 (Glass)	2–3	near end	near centromere	52 :	41 : 6		
V4 (Glass)	2–3	near end	near centromere	55 :	45 : 0+		
V5 (Glass)	2–3	near end	near centromere	50 :	48 : 3		
V5/Grape (Glass)	2–3×2–3	near end both	near centromere (In L×In R)	61 :	17 : 18 : 4		
No. 8 Brown	3–4	L 0.23	—	60 :	32 : 8	48	58
36 Brown	3–4	L 0.01	—	70 :	27 : 3	53	61
31 Brown	3–4	L 0.0	—	68 :	28 : 4	52	70
27 Brown	3–4	R 0.02	—	72 :	25 : 3	47	64
9 Brown	3–4	R 0.35	—	59 :	39 : 2	47	60
2 Brown	3–4	R 0.68	—	52 :	47 : 2	48	62

Of the 2-3 translocations, A and C reported by DOBZHANSKY and STURTEVANT (1931) and the one by PIPKIN (1940) showed relatively high frequencies of both types of adjacents while E showed only one type. As in the corn translocations these differences are associated with the presence of short vs. long interstitial segments. PIPKIN, however, based on calculations of chiasma frequency from the genetic data, concluded that the adjacent 2 segregations came from chain configurations. The chain-forming corn translocations reported here had few or no adjacent 2 segregations. In maize, similar data are needed on chain-forming translocations not involving chromosome 6. Although translocation B had a longer interstitial segment, little crossing over is expected in it; hence it appears to be an exception since it produced only one type of adjacent. Also in A, C and in PIPKIN's 2-3 translocations there was a definite excess over 50 percent of alternate segregation (B was high also, but was also higher than the others in adjacent 1). In Drosophila, if both translocated pieces are long as in these, the greater opportunity for crossing over in them seems to permit the ring to take a zigzag orientation more frequently. This does not occur consistently if at all in the corn translocations reported here. These in corn may not be comparable, since only one interstitial segment was long. The translocations reported by GLASS (1935) had at least one long

interstitial segment and a few adjacent 2 segregations, agreeing with the results for the corn translocations of similar type. In those reported by GLASS, chain configurations should have been frequent, another possible reason for few adjacent 2 segregations. In his experiments the female parent carried inversions in chromosomes 2 and 3 while the male did not. The V5/Grape cross gave different results, interpreted as being due to an effect of equal axis lengths, but it was also the only cross in which both parents carried inversions. Such a use of inversions to "eliminate crossovers" is of doubtful value, since in many cases only the genetic recombinations are eliminated, cytological crossing over approaching normal or only somewhat reduced (BEADLE and STURTEVANT 1935).

A meiocyte with single crossovers or 3-strand doubles in the interstitial segment produces simultaneously in equal proportions the four types classified genetically (by the method used in Drosophila) as resulting from alternate as well as adjacent 1 segregation. This is true whether the chromosome segregation following these crossovers is all alternate or 1 alternate:1 adjacent (see fig. 1A, B). The actual type of segregation cannot be determined from these Drosophila or maize experiments for those with long interstitial segments.

Those reported by BROWN (1940) involved the very short chromosome 4, and may belong to a special class. Here two adjacent arms of the possible 4-armed cross in the heterozygote were short. Adjacent 2 segregation was low in all (only one with as much as 8 percent), but there was a wide range of alternate segregation. The shorter the translocated piece of 3 (likewise the longer the interstitial segment) the nearer to 1:1 was the ratio of alternate:adjacent 1 segregation. This latter may be due to the greater frequency of crossover tetrads as pointed out above. As the translocated piece increased in length (the shorter the interstitial segment) the frequency of alternate segregation increased and that of adjacent 1 decreased, the extreme being 72 percent of alternate and 25 percent of adjacent 1. BROWN suggested that this may be explained if at pachytene the two parts of chromosome 3 pair with the normal 3 and segregate from it (the same result as from alternate segregation). The more nearly equal are the translocated and non-translocated pieces, the higher the expected frequency of this behavior. Translocations of this type might be expected to behave differently from rings, chains with one short translocated piece, or chains and "pairs" resulting from two short translocated pieces (alternate arms of this latter "cross" at pachytene would be short). She also determined that in the adjacent 1 type in comparison with the completely disjunctional types, crossing over was greatly reduced in the translocated piece, this being the arm which underwent non disjunction. Those with crossovers in this arm were therefore more likely to be followed by alternate segregation.

The interpretation of the results from mating translocation heterozygotes might be complicated in Drosophila by the fact that there is no crossing over in the male. Both sexes must produce the same class of adjacents if there are to be offspring of that class. The calculations assumed similar frequencies in

the two sexes. If the relation between chromosome segregation and crossing over holds, then in the male Drosophila with no crossing over all translocations should produce both kinds of adjacents and with similar frequencies. The segregation in the female should differ depending on the crossover length of the interstitial segment. Egg counts from reciprocal crosses between normals and translocation heterozygotes might give some information on this point, but there are only a few published data for reciprocals (see above summary). In that case, differences between translocation heterozygotes when tested as males should not be expected, but the egg counts on DOBZHANSKY and STURTEVANT's A and C did differ from those on B in crosses of N with T/+males. In PIPKIN's *T2-3*, there was a relatively small difference between tested males and females, and none would be expected. In BROWN's translocations, the tests of males showed uniform values of about 50 percent mortality. In the tests of females there was some variation, but all showed higher percentages of survival. This seems to indicate differences at least in this class of translocations in segregation between male and female translocation heterozygotes. As suggested by STURTEVANT, the earlier work on Drosophila translocations may have been complicated by other factors, as for example inversions, the high frequency of which was not realized until the salivary chromosome technique was discovered.

One unsolved problem is that posed by certain plant species which regularly show alternate segregation in translocation rings. In Datura, BLAKESLEE et al. (1937 and earlier) have reported rings with and without sterility. In Oenothera, as CLELAND (1936, p. 326) states it, at meiosis "adjacent chromosomes are separated to opposite poles in a very large majority of the cells," and (1929, p. 319) that even in the 10 or 20 percent having irregularities most of the chromosomes are arranged in the zigzag manner at metaphase I. It is also apparent that those Oenothera translocations which have survived in evolution have short interstitial segments, either physically short or if longer have little crossing over due to heterochromatin (MARQUARDT 1948). EMERSON (1936) has shown that certain of them do have differential segments which are short in terms of genetic crossing over, others have no such segment (or at least extremely short). Since no marked differences in chromosome length are apparent even in the large rings, it has been concluded that the pieces translocated have been of approximately equal length. This, combined with the lack of differential segments in the larger rings indicates that the breaks are nearly median in the translocations which have survived (the centromeres are also approximately median). That translocations with a long interstitial segment may occur in Oenothera has been shown by X-ray treatment. CATCHESIDE (1935), by X-ray treatment of *Oe. blandina*, an *Oe. Lamarckiana* segregate with seven pairs, produced two such translocations, B having 23 percent and E 49 percent of pollen abortion. He suggested that the abortion might be due to a pollen lethal, but translocation B was later reported to have been established in homozygous condition (CATCHESIDE 1939), E was not (8 normals tested according to a private communication). According to his published report (1935), limited observations indicated alternate orientation in all. If this is

true in spite of differences in breakage point positions, then it must be concluded that genes control the type of orientation. In *Oe. Hookeri*, MARQUARDT (1948) reported X-ray induced translocations with frequent chain configurations and "pairs" accompanied by pollen abortion. He also noted that crossing over in interstitial segments results in quartets with 50 percent spore abortion even with alternate segregation.

SMITH (1948) has pointed out also that zigzag configurations in the rings showing crossing over in the interstitial segment would still result in 50 percent of ovule or pollen abortion. To extend this further, it would be possible for a species to have only alternate segregation and yet approach 50 percent of pollen abortion if most sporocytes had at least one crossover in the interstitial segment, assuming that chromosomes that cross over pass to opposite poles. The single crossovers and 3-strand doubles give this result directly. With alternate segregation 2-strand doubles result in quartets with all normal spores and the 4-strand doubles result in quartets with all abortive spores, but the two together should give 50 percent abortion if they are in the random 1:1 ratio (fig. 1B).

In the reports on translocations in other species there is no information on the lengths of the interstitial segments. Only a few which attempted to analyze the segregations are considered here.

In *Tradescantia edwardsiana* SAX and ANDERSON (1933) reported a translocation in which rings were rare and 15 percent were separate pairs. As in Drosophila, chiasmata at the ends (or in the translocated pieces) in the ring configurations were more likely to permit an alternate orientation of the chromosomes. A translocation in *T. reflexa* with rings more frequent than chains had 83 percent of adjacent segregation. They also reported data on the Rhoeo ring indicating only 21 percent of alternate orientation, the remainder being made up of different types of single and double non disjunction. In a translocation complex of four chromosomes in *Campanula persicifolia*, GAIRDNER and DARLINGTON (1931) reported the percentage of alternate orientations was somewhat in excess of 50, but both types of adjacents occurred in rings as well as in chains.

SMITH (1936) reported a ring of four chromosomes in a cross of *Triticum monococcum* × *T. aegilopoides*. In F_2 most of the normals as well as the ring-formers had less than 10 percent of aborted pollen. At late metaphase, he reported "open" and "zigzag" configurations in about a 1:1 ratio. Sterility as low as that is difficult to explain, since the "open" rings would be expected to result in abortive combinations, unless previous crossing over had occurred in an interstitial segment, and then would not be expected to be lower than 25 percent.

In an unequal translocation, SMITH (1939) reported only 11 percent sterility with possibly 93 percent of zigzag orientation as indicated by the frequency of deficiency-duplication plants.

THOMPSON and THOMPSON (1937) and THOMPSON and HUTCHESON (1942) reported seven different translocations in *T. monococcum* and two in *T. durum*, all of which had very low pollen abortion. Three of those in *monococcum* had

three to eight percent more pollen abortion than did the normals. The majority were described as having equal arms in the pachytene cross and long pieces exchanged, consequently short interstitial segments. One with a short translocated piece (probably indicating a long interstitial segment) was reported to have low sterility, but the amount was not reported.

In barley (the Mars variety of *H. vulgare*), only plants with pollen sterility (descendants of X-rayed seeds) have been examined cytologically (BURNHAM unpublished). Those with a single ring of four chromosomes vary in pollen abortion from about 14 to 58 percent. The range in sterility might be due to differences in the proportions of open and zigzag rings, or to differences between them in the amount of crossing over dependent on differences in length of the interstitial segments. If barley has a genotype for regular zigzag orientation at metaphase I, rings with little or no sterility would be expected, i.e. those with genetically short interstitial segments. Lines with normal pollen, sibs of those in which the translocations were first identified, are being examined but thus far all have had 7 pairs of chromosomes. One of the points of difference between the "Maize" and "Oenothera" types of ring behavior listed by THOMPSON and HUTCHESON (1942) is that in corn there are numerous intercalary chiasmata while in Oenothera and wheat most are terminal. This does not appear to be true of all the corn chromosomes. They interpreted a statement of ANDERSON's that by diakinesis only the ends of the chromosomes are associated as meaning that "open" flat rings are characteristic of diakinesis in maize translocations, implying an earlier and a different type of opening out than in wheat. However, zigzag and open rings are found at diakinesis in maize, as shown in table 4 in this paper. Another point of difference listed is that in most chromosomes of maize the centromeres are not median and the chromosomes are long; while in wheat and Oenothera the centromeres are median and the chromosomes are short. In corn, chromosomes 1 and 5 which are nearly equal-armed, are involved in several translocations with breaks at widely diverse points yet all appear to show the usual 50 percent of pollen abortion. A similar test in the short chromosomes (e.g. 9 and 10) of corn is lacking. In the two *T5-6c* stocks reported here, pollen abortion remains the same although segregation is very different in the two stocks.

A search for genes which may bring about directed segregation is being made in crosses between maize of different origins and a tester translocation with short interstitial segments.

Data from plants heterozygous for *T5-6c* and also for *In5a* will be reported later.

SUMMARY

1. This paper presents a study of chromosome segregation in 27 translocation heterozygotes involving chromosome 6 in corn. Certain segregations can be recognized by the nucleolar make-up of the spore quartets or by differences between predicted and observed pollen abortion. In translocations with the break in the short arm of chromosome 6, crossing over in the interstitial segments results in a distinctive quartet type.

2. Three translocation heterozygotes, (*2-6a*, *6-10b*, and *T5-6c* homozygous

for inversion *5a*) with short interstitial segments (1.0, 5.3 and 10.6 percent respectively of crossover type quartets), had the following percentages of alternate: adjacent 1 (homologous centromeres disjoin): adjacent 2 (non disjunction of homologous centromeres): for *T2-6a*, 48:26:26; for *T6-10b*, 58:19:23; for *T5-6cIn5a*, 50:31:19.

3. For individual plants in *T5-6cIn5a*, there was a high negative correlation ($r = -0.77$) between the percentage of crossover type quartets and the frequency of adjacent 2 segregation.

4. Two translocation heterozygotes, *T4-6* Li and *T5-6c*, had a long interstitial segment (63 percent of crossover quartets in each), and very little adjacent 2 segregation. The difference between *T5-6c* and *T5-6cIn5a* was only in the length of the interstitial segment, axis lengths and arm lengths of the pachytene "cross" remaining the same.

5. For translocations with the break in the long arm of chromosome 6:

a. A group of ten with short interstitial segments had 14 to 36 percent of adjacent 2 segregation, the average being 25.

b. A group of nine with at least one long interstitial segment had an average of 5.4 percent of adjacent 2 segregation. One, *T6-8a* with 17.4 percent, overlapped the other group.

6. Four chain-forming translocations, two with short and two with long interstitial segments, had very low frequencies of adjacent 2 segregation.

7. In *T5-6c*/+ with a high percent of crossover quartets having two normal and two abortive spores, ovule and pollen abortion were about equal indicating no megaspore competition.

8. Relative lengths of the two axes is not a factor determining segregation. The segregations are similar whether the centromeres are on the shorter or the longer axis.

General Conclusions

1. In corn, at least for translocations involving chromosome 6, the following relationships between crossing over in interstitial segments and chromosome segregation in translocation heterozygotes are found:

a. *In rings*, low crossover frequency (short segments) is accompanied by a high frequency of adjacent 2; while high crossover frequency (long segment) is accompanied by a low frequency of adjacent 2 (little or none). The evidence indicates that when chromosomes cross over in the interstitial segment they pass to opposite poles, and that adjacent 2 segregation occurs only when there has been no crossing over.

b. In chains, on the other hand, little or no adjacent 2 segregation occurs in any of the translocations, no matter how short the interstitial segment.

2. It is suggested that directed segregation in translocation heterozygotes may be genetically controlled, the amount of spore abortion in such a species depending on the frequency of crossing over in the interstitial segment. Genetic recombination in such a species as pointed out by LAMM (1948; quoting Tometorp) may be greatly reduced, since single crossovers and certain of the multiples are not recovered following alternate segregation. This does

not account for all the reduced crossing over in corn translocations, since there is no evidence of any great amount of directed segregation. In the search for such genes in corn, translocations with short interstitial segments should be used.

LITERATURE CITED

ANDERSON, E. G., 1938 Translocations in maize involving chromosome 9. Genetics **23:** 307–313.

1939a Translocations in maize involving chromosome 8. Genetics **24:** 385–390.

1939b Maize Genetics Coöperation News Letters.*

1940 Maize Genetics Coöperation News Letters.*

ANDERSON, E. G., and L. F. RANDOLPH, 1945 Location of the centromeres on the linkage maps of maize. Genetics **30:** 518–526.

BEADLE, G. W., and A. H. STURTEVANT, 1935 X-chromosome inversions and meiosis in *Drosophila melanogaster*. Proc. nat. Acad. Sci., Wash. **21:** 384–390.

BLAKESLEE, A. F., A. D. BERGNER, and A. G. AVERY, 1937 Geographical distribution of chromosomal prime types in *Datura Stramonium*. Cytologia (Fujii Jub. Vol.) 1070–1093.

BRIEGER, F. G., 1945 Competicao entre megasporios em milho. An. Esc. sup. Agric., Sao Paulo. Univ. **2**(18): 239–267.

BROWN, M. S., 1940 The relation between chiasma formation and disjunction. Univ. Texas Publ. **4032:** 11–64.

BURNHAM, C. R., 1932a The association of non-homologous parts in a chromosomal interchange of maize. Froc. 6th Int. Cong. Genetics **2:** 19–20 (abstract).

1932b An interchange in maize giving low sterility and chain configurations. Proc. nat. Acad. Sci., Wash. **18:** 434–440.

1945 Chromosome disjunction in maize interchanges. Genetics **30:** 2 (abstract).

1948 Chromosome segregation in translocations involving chromosome 6 in maize. Genetics **33:** 605 (abstract).

1949 Chromosome segregation in maize translocations in relation to crossing over in interstitial segments. Proc. nat. Acad. Sci., Wash. **35:** 349–356.

CATCHESIDE, D. G., 1935 X-ray treatment of Oenothera chromosomes. Genetica **17:** 313–341.

1939 A position effect in Oenothera. J. Genet. **38:** 345–352.

CLARKE, A. E., and E. G. ANDERSON, 1935 A chromosomal interchange in maize without ring formation. Amer. J. Bot. **22:** 711–716.

CLELAND, R. E., 1929 Meiosis in the pollen mother cells of the Oenotheras, and its probable bearing upon certain genetical problems. Proc. int. Cong. Plant. Sci **1:** 317–331.

1936 Some aspects of the cytogenetics of Oenothera. Bot. Rev. **2:** 316–348.

DARLINGTON, C. D., 1937 Recent Advances in Cytology. xvi+671 pp. Philadelphia: P. Blakiston's Son & Co.

DOBZHANSKY, TH., and A. H. STURTEVANT, 1931 Translocations between the second and third chromosomes of Drosophila and their bearing on Oenothera problems. Carnegie Inst. Wash. Pub. #421: 29–59.

EMERSON, R. A , G. W. BEADLE, and A. C. FRAZER, 1935 A summary of linkage studies in maize. Cornell Univ. agric. Exp. Sta. Mem. **180:** 83 pp.

EMERSON, S., 1936 A genetic and cytological analysis of *Oenothera pratincola* and one of its revolute-leaved mutations. J. Genet. **32:** 315–342.

GAIRDNER, A. E., and C. D. DARLINGTON, 1931 Ring formation in diploid and polyploid *Campanula persicifolia*. Genetica **13:** 113–150.

GLASS, BENTLEY, 1935 A study of factors influencing chromosomal segregation in translocations of *Drosophila melanogaster*. Univ. Mo. Research Bulletin **231:** 28 pp.

HALDANE, J. B. S., and H. L. K. WHITEHOUSE, 1944 Symmetrical and asymmetrical postreduction in ascomycetes. Nature **154:** 704.

HANSON, W. D., and H. H. KRAMER, 1949 The genetic analysis of two chromosome interchanges in barley from F_2 data. Genetics **34:** 687–700.

* Permission to use this unpublished information has been generously granted.

KOLLER, P. C., 1944 Segmental interchange in mice. Genetics **29:** 247–263.

LAMM, R., 1948 Linkage values in an interchange complex in *Pisum*. Hereditas **34:** 280–288.

LONGLEY, A. E., 1948 Maize Genetics Coöperation News Letter 22.*

MARQUARDT, H., 1948 Das Verhalten röntgeninduzierter Viererringe mit grossen interstitiellen Segmenten bei *Oenothera Hookeri*. Z.I.A.V. **82:** 415–429.

MCCLINTOCK, B., 1934 The relation of a particular chromosomal element to the development of the nucleoli in *Zea Mays*. Z. Zellforsch. **21:** 294–328.

1941 Spontaneous alterations in chromosome size and form in *Zea Mays*. Cold Spring Harbor Symp. Quant. Biol. **9:** 72–80.

1945 Neurospora I. Preliminary observations of the chromosomes of *Neurospora crassa*. Amer. J. Bot. **32:** 671–678.

PIPKIN, S. B., 1940 Segregation and crossing over in a 2, 3 translocation in *Drosophila melanogaster*. Univ. Texas. Publ. **4032:** 73–125.

RHOADES, M. M., 1941 Different rates of crossing over in male and female gametes of maize. J. Amer. Soc. Agron. **33:** 603–615.

ROBERTS, L. M., 1942 The effects of translocation on growth in *Zea Mays*. Genetics **27:** 584–603.

SAX, K., 1932 The cytological mechanism of crossing over. J. Arnold Arboretum **13:** 180–212.

SAX, K., and E. ANDERSON, 1933 Segmental interchange in chromosomes of Tradescantia. Genetics **18:** 53–67.

SMITH, L., 1936 Cytogenetic studies in *Triticum monococcum* L. and *T. Aegilopoides* Bal. Mo. agric. Exper. Sta. Res. Bull. 248.

1939 Reciprocal translocations in *Triticum monococcum*. Genetics **24:** 86.

1948 A haplo-viable deficiency-duplication from an interchange in *Triticum monococcum*. Bot. Gaz. **109:** 258–268.

STURTEVANT, A. H., and T. DOBZHANSKY, 1930 Reciprocal translocations in Drosophila and their bearing on Oenothera cytology and genetics. Proc. nat. Acad. Sci. **16:** 533–536.

THOMPSON, W. P., and MARY G. THOMPSON, 1937 Reciprocal chromosome translocations without semi-sterility. Cytologia (Fuji Jub. Vol.) 336–342.

THOMPSON, W. P., and I. HUTCHESON, 1942 Chromosome behaviour and fertility in diploid wheat with translocation complexes of four and six chromosomes. Canad. J. Res. **20:** 267–281.

WEINSTEIN, A., 1929 Four-strand crossing over. Anat. Rec. **41:** 109–110.

1936 The theory of multiple-strand crossing over. Genetics **21:** 155–199.

* Permission to use this unpublished information has been generously granted.

SELF-INCOMPATIBILITY STUDIES IN GUAYULE
II. INHERITANCE*

D. U. GERSTEL[1]

U. S. Department of Agriculture, Salinas, California

Received January 11, 1950

THE inheritance of self-incompatibility in diploid guayule (*Parthenium argentatum* Gray) can be studied with relative ease because crosses are either compatible or fully incompatible; no kind of pseudofertility obscures the clear-cut results as in so many other species (GERSTEL and RINER 1950). Furthermore, guayule is a perennial shrub and several generations can be made to flower at the same time. This species also offers a novel type of self-incompatibility which differs basically from the widespread scheme found in *Nicotiana alata* and from those of almost all other investigated species (reviewed by STOUT 1938), as will be shown in the following.

This became clear at the onset of the present study when it turned out that certain mother-progeny pairs, then available, were reciprocally cross-incompatible. Because these findings could not be reconciled with a system of a single series of oppositional alleles of the nature found in Nicotiana, Trifolium and others, the problem was studied in detail.

METHODS AND MATERIALS

The incompatibility reactions reported in this paper were mostly observed by means of microscopic examination of pollen tubes in stigmata one to two hours after pollination. They were stained in lactophenol with acid fuchsin and light green. For a detailed account of the techniques employed and a description of the behavior of compatible and incompatible pollen see GERSTEL and RINER (1950). It was found that after compatible crosses pollen tubes can be located in the stigmata while they fail in the incompatible ones.

Seed fertility counts (table 3) were based on the number of achenes found to contain filled seeds after soaking and dissection. This criterion, rather than germination figures, was employed because the latter are highly variable in guayule and subject to physiological complications; the percentage of filled achenes gives a good estimate of potentially germinable seed (BENEDICT and ROBINSON 1946). Many of the filled seed samples reported were germinated, however, and gave fair to good germination.

No emasculation was practised as in all previous work on self-incompatibility it was concluded that compatible pollen of the *same* species[2] does not stimulate

* Part of the cost of the accompanying tables has been paid by the GALTON AND MENDEL MEMORIAL FUND.

[1] Formerly Associate Geneticist, U. S. NATURAL RUBBER RESEARCH STATION, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. DEPARTMENT OF AGRICULTURE, Salinas, California. The work was completed while the author was a Research Fellow at the CALIFORNIA INSTITUTE OF TECHNOLOGY.

[2] Not so for pollen of "fairly closely related species" which in Madia apparently stimulates the own pollen of a self-incompatible plant to function (CLAUSEN, KECK and HIESEY 1945). Ob-

incompatible pollen to function. In guayule, crosses between an unemasculated homozygous recessive "xanthous" type and "normal" as the male also indicated that there is no stimulation of incompatible by compatible pollen within the species (unpublished).

The diploid (2n = 36) parent plants numbered SP-1 to SP-15 were selected from a planting made by the late DR. W. B. MCCALLUM, in which they occurred intermingled with polyploid plants. They were dug and planted in movable wooden boxes. It is not known to what extent these plants were

TABLE 1

Compatibilities and incompatibilities in crosses between sibs ex SP-8×SP-7 and their parents.

♀ \ ♂	SP	SP	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75	
	7	8	2	3	6	10	11	15	19	5	8	9	12	18	1	7	13	14	16	20	4	17	
SP-7	−	+	−	−	−	−	−	−	−		−		−	−	−	−	−	−	−	−	−	−	P
SP-8	+	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+		+	+	+	+	
75-2	+	−	−		−					−	−	−			+	+	+					+	
75-3	+	−		−	−		−	−	−	−	−			−	+	+	+	+	+		+		
75-6		−	−	−	−	−	−		−	−	−			−		+	+			+		+	
75-10	+	−			−	−	−	−				−	−	−	+	+	+	+		+	+	+	A
75-11	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+		+	+	+	
75-15	+	−		−		−	−	−			−		−		+		+	+		+		+	
75-19	+	−		−	−		−		−	−				−			+		+	+		+	
75-5		+	−	−	−		−		−	−	−			−	+		+		+		+	+	
75-8	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+		+	+	+	+	
75-9	+	+	−			−	−				−	−	−	−			+	+	+	+		+	B
75-12	+	+					−	−			−	−			+		+	+			+	+	
75-18	+	+			−	−	−		−	−	−			−	+	+	+	+		+		+	
75-1	−	−	−	−		−	−	−		+	+		+	+			−	−	−	−	−	−	
75-7	−	−	−	−	−	−	−				+			+	−	−	−	−					
75-13	−	−	−	−	−	−	−	−	−	+	+	+	+	+	−	−	−	−		−	−		
75-14	−	−		−		−	−					+	+	+	−	−	−			−		−	C
75-16	−	−		−			−		−	+	+	+			−				−	−		−	
75-20	−	−			−	−	−	−	−		+	+		+	−		−	−	−	−		−	
75-4	−	+		+		+	+			+			+		−		−				−	−	
75-17	−	+			+	+	+	+	+	+	+	+	+	+	−		−	−	−	−	−	−	D
	P		A							B					C						D	

− Entries denote that of at least seven stigmata pollinated, none showed any tubes (very exceptionally 1 or 2 tubes occurred, contaminants or mutants).

+ Entries were made after at least seven stigmata had shown tubes in any given cross. (The minimum of tubes was three, but almost always their number was too large to be counted. Failures, in addition to seven positive results occurred).

genetically related to each other, nor from which part of the "guayule belt" of Texas or Old Mexico their progenitors had come. The diploids IV-P and IV-F were a mother-offspring pair from an accession (A-4255) collected by

servations in the following genera may warrant a similar interpretation: Veronica (ZÄMELIS and MELDERIS 1932), Galium (FAGERLIND 1937), Taraxacum (GUSTAFSSON 1937), Primula (ERNST 1944), and Calamagrostis (NYGREN 1946). The behavior of guayule is under investigation.

DR. LEROY POWERS in the State of Durango, Mexico. The mother, IV-P, was homozygous recessive for "xanthous" and the offspring heterozygous and thus presumably an outcross with an unknown normal male (GERSTEL 1947).

In the progeny cultures from the crosses SP-7×SP-8 and its reciprocal (tables 1–4) all the survivors were studied and no selection exercised. Tetraploids were obtained from the cross SP-8×SP-7 by colchicine treatment (described by GERSTEL and RINER 1950). There were 82 survivors of the treatment. The first ten tetraploids to come into bloom were used for incompatibility analysis (tables 4, 5). About six more tetraploids were recognized later, the remaining plants being diploids, but their incompatibility reactions were not studied. The chromosome numbers of the ten plants used were ascertained by PMC smears to be 72 in each instance and throughout the pollinations all pollen was carefully measured to avoid the diploid branches which some of the plants possessed.

For the second generation from SP-8×SP-7 (table 7) a greater number of plants was grown in flats, but only 12 plants from each culture were transferred to large crocks for analysis (some died later; 13 plants used in one instance). Selection was avoided by using solid blocks of plants from the flats. Exceptions were nine albinos which eliminated themselves from a number of flat cultures before transplanting (in cultures 94, 98, 100, 116) and two dwarfs which were potted separately and then bloomed only sparingly, with abnormal flowers, during the testing period (culture 94). The remaining surplus was kept in a dormant state and several replacements were drawn from this reserve.

In the two reciprocal progenies from parents SP-3 and SP-6 (table 8) a number of plants were picked for use without any intended selection but also without regard for randomization procedures.

Therefore, the cultures were not entirely random lots, but it appears improbable that the composition of the cultures with respect to their incompatibility factors was affected.

RESULTS

Plants SP-7 and SP-8 were grown together in an isolated plot at STANFORD UNIVERSITY in 1947. In the late fall of 1947 progeny cultures were grown of seed harvested from these two plants representing the reciprocal crosses SP-7×SP-8 and SP-8×SP-7.

Family SP-8×SP-7

The most intensely studied progeny was from the cross SP-8 ♀ ×SP-7 ♂ (table 1, culture 75). Eighteen of the twenty sibs were tested for self-incompatibility and in each case their own pollen failed on their stigmata. Next, practically all twenty sibs were tested reciprocally with both parents. The results clearly indicated the existence of four classes:

Pollen of the parent SP-7 produced tubes in the stigmata of all the plants placed in classes A and B, but the pollen of A and B failed in the reciprocal cross back to SP-7. Members of these two classes could be distinguished, how-

ever, from each other in tests with the SP-8 parent; A×SP-8 were incompatible in reciprocal tests while B×SP-8 were reciprocally compatible.

Classes C and D were not crossable with SP-7 either way, but could be separated by testing with SP-8: C pollen functioned on SP-8, but SP-8 pollen failed on C stigmata; D was reciprocally compatible with SP-8.

TABLE 2

Number of plants in each incompatibility group

CROSS	A	B	C	D	TOTAL
SP-8×SP-7	7	5	6	2	20
SP-7×SP-8	3	3	1	3	10
4n(SP-8×SP-7)	2	1	6	1	10
Total	12	9	13	6	40
Chi square=3.0		df=3		.5>P>.3	

By this method seven sibs were placed in class A, five in B, six were C, and two D (table 2; the chi square test, applied to the total from this and two additional cultures, indicated that the classes could be equal). Subsequent tests (table 1) revealed that sibs belonging to the same class were always cross-incompatible. There were also cross-incompatibilities between certain classes. All members of class A were incompatible with all members of class B, and, similarly, members of class C were incompatible with those of D.

Crosses between members of the following classes were found to be reciprocally compatible: A with D; B with C and with D. Crosses between classes A and C gave different results, depending on which parent was the male: if pollen from A plants was used on C stigmata no tubes resulted, whereas they did in the reciprocal crosses.

Practically all possible cross-pollinations between parents and sibs and between sibs were made, but only those cases where at least seven positive results or seven negative results had been obtained were entered in table 1. The units for these counts were the individual stigmata, and as a flower head has five stigmata this insured that at least one repetition of each test had been made. The number of tubes required for "+" were at least three in a given stigma, but usually their number was very much larger. Failures and low tube counts occurred quite frequently in crosses between compatible plants, in addition to the positive results, but their distribution was erratic and therefore not indicative of any "weak" compatibilities.

Very rarely one, or still more rarely two tubes could be observed in the crosses tabulated as "−"; these may easily be ascribed to contamination or perhaps mutation. Among the large number of tests which were not entered in table 1 because their number fell short of seven none gave positive results where negative ones were expected.

Crosses to yield seed (table 3) were made between all but one of the combinations which had given positive pollen tube tests and were therefore ex-

TABLE 3

Percentages of filled achenes from crosses between members of the various classes and some selfings.

♀ \ ♂	SP-7	SP-8	A	B	C	D
SP-7	0.4	64.0			0.0	
SP-8	72.0	0.0		46.5	*56.1*	*51.0*
A	*69.0*			0.0	*21.3*	
B	*55.0*	47.4	0.0		*55.2**	38.3
C		*14.5*	1.2	51.0		
D		79.1*	*65.0*	*54.1*		

* Size of the samples varied between 100 and 300, with exception of those marked *; these varied between 40 and 60 achenes.

Italicized figures designate seed samples from which plants were used for the second generation tests (table 7).

Framed fields denote crosses which had given + pollen tube tests; unframed fields − tests.

TABLE 4

Pollen-tube tests of progeny SP-7×SP-8.

♀ \ ♂	SP 7	SP 8	74 3	74 17	79 3	74 1	74 5	79 4	74 6	74 2	79 1	79 2	
SP-7	−	+	−	−	−	−		−	−	−	−	−	P
SP-8	+	−	−	−	−	+	+	+		+	+		
74-3	+	−		−		−			−	+			A
74-17	+	−	−				−		+	+			
79-3	+	−				−	−				+	+	
74-1	+	+			−	−		−	+		+		B
74-5	+	+		−	−					+			
79-4	+	+								+			
74-6	−	−	−	−	−	+	−						C
74-2	−	+	+	+			+	+	−		−		D
79-1	−	+				+				−		−	
79-2	−	+			+	+		+			−		
	P		A			B			C	D			

Pollinations between parent plants and progeny were classified according to the same criteria as used for SP-8×SP-7 (at least seven failures and seven successes). Relations between sibs were entered on the basis of results from single heads only (five failures or five successes).

pected to be fertile; also included were several combinations not expected to yield seed on the basis of the pollen tube tests. These crosses were made either under paper bags in the greenhouse or by isolating paired plants in various parts of Central California. Only one plant from a class was used for a particular cross. The data in table 3 represent the percentages of filled seeds obtained and they correlate reasonably well with expectation; filled achenes ranging from 21.3 percent to 79.1 percent were obtained where expected. This wide range was probably due to the extremely variable conditions under which the seed was produced. On the negative side, no filled achenes were obtained from selfing of SP-8 and in crosses between SP-7×C, A×B and B×A as expected. One lone seed was unexpectedly obtained from selfing SP-7 and two from C×A; the cross C×SP-8 yielded 14.5 percent filled seed contrary to expectation. This is less, however, than the filled percentages obtained in any of the positive crossses.

Family SP-7×SP-8

Ten plants (cultures 74 and 79) of this cross were available for study and the same four classes as in the reciprocal cross were found here. Table 4 shows the classes A (3 plants), B (3), C (1), and D (3) having the same cross relations with parents and sibs already known from SP-8×SP-7. The same rigorous criteria were applied as before in tests between parents and progeny; that is, "+" entries were based on at least seven stigmata with tubes (three or more) and "−" results were based on seven failures (no tubes or very rarely one or two).

In testing relations between sibs only single flower heads were used and the entries were based on five successes or five failures of pollen tubes to grow. With this less rigorous criterion occasional "−" signs can be found in the table where "+" would be expected.

A few tests between members of the reciprocal progenies SP-8×SP-7 and SP-7×SP-8 were also made; they confirmed in every case the identity of the reciprocal crosses; their results were not tabulated, however.

Tetraploids

The tetraploid plants (culture 76) obtained by colchicine treatment of sib-seedlings of the progeny SP-8×SP-7 were also tested for self-incompatibility relations with parents and among sibs. As table 5 indicates, these tetraploids were self-incompatible and fell into exactly the same incompatibility classes as their diploid sibs, with class A containing two; B, one; C, five; and class D, one plant (table 2). All the possible parent-progeny tests were made, but only a part of the possible sib-combinations were tested and some of the entries were based on results from single flower heads, but the conclusions which can be drawn from table 5 are unequivocal: the tetraploids do behave the same as the diploids in this family.

Second generation cultures from SP-8×SP-7

A working hypothesis attempting to explain the methods of control and inheritance of self- and cross-incompatibilities was elaborated on the basis of the

results of the pollen tube tests available in the fall of 1948 presented in the foregoing. The derivation of this hypothesis is described in detail under "Discussion." For the sole purpose of guiding the reader through the following experiments, which were intended to test the working hypothesis, the features of this hypothesis are anticipated and put down as simple postulates (cf. fig. 1):

1) Inheritance of self-incompatibility is controlled by a series of multiple alleles. Certain pairs of alleles were assigned to the various phenotypes;

TABLE 5

Compatibilities in a tetraploid family ex SP-8×SP-7 (4n progeny produced with colchicine from 2n parents).

♀ \ ♂	SP 7	SP 8	76 10	76 26	76 7	76 8	76 27	76 63	76 6	76 28	76 62	76 43	
SP-7	−	+	−	−	−	−	−	−	−	−	−	−	P
SP-8	+	−	−	−	+	+	+	+	+	+	+	+	
76-10	+	−	−			+	+	+			−	+	A
76-26	+	−		−	−				+	+	+		
76-7	+	+		−	−					+			B
76-8	−	−	−			−							C
76-27	−	−	−				−					−	
76-63	−	−	−					−					
76-6	−	−		−					−				
76-28	−	−		−	+					−	−		
76-62	−	−	−	−						−	−	−	
76-43	−	+	+				−				−	−	D
	P		A		B	C						D	

Some of the entries were based on single heads (at least 4 stigmata), but most entries were based on several heads.

namely, SP-7 = R_1R_2; SP-8 = R_3R_4; A = R_1R_3; B = R_1R_4; C = R_2R_3, and D = R_2R_4.

2) Control of incompatibilities is sporophytic; that is, the two alleles of the pollen parent determine together the reaction of all its pollen grains; these act alike regardless of which allele a particular grain contains.

3) R_4 is recessive to the other three alleles and R_2 is dominant over them; R_1 and R_3, the intermediates, do not show dominance over each other.

To test the validity of the hypothesis, predictions were made regarding the progeny classes expected from crosses of members of the sib-classes A, B, C, and D from backcrosses to their parents. As pollen tube tests are a very time-consuming task, only a selected number of progenies were tested in the summer of 1949 from crosses made in the fall of 1948. These included two where the

parents, according to the hypothesis, had in common no allele of the postulated series of self-incompatibility alleles R_1, R_2, R_3, and R_4. These two cultures represented crosses between the classes B×C and D×A (plants used were 75-9×75-1 for the former and 75-17×75-15 for the latter). Two progenies were also studied from sibs which were postulated to have one common allele, A×C and D×B (75-10×75-13 and 75-17×75-8). Five progenies were grown from backcrosses: A×SP-7, B×SP-7, C×SP-8, SP-8×C, and SP-8×D (plants 75-2, 75-8, 75-14, 75-14, and 75-17 were the sibs used).

TABLE 6

Expected reactions between testers (top row) and all the possible classes.

CLASS	GENOTYPE	B	D	SP-7	SP-8	
		R_1R_4	R_2R_4	R_1R_2	R_3R_4	
			Heterozygotes			
A	R_1R_3	−−*	++	+−	−−	
B	R_1R_4	−−	++	+−	++	
C	R_3R_3	++	−−	−−	−+	
D	R_2R_4	++	−−	−−	++	
SP-7	R_1R_2	−+	−−	−−	++	
SP-8	R_3R_4	++	++	++	−−	
			Homozygotes			
	R_1R_1	−−	++	+−	++	indistinguishable from B
	R_2R_2	++	−−	−−	++	indistinguishable from D
	R_3R_3	++	++	++	−−	indistinguishable from SP-8
	R_4R_4†	++	++	++	++	

* First symbol for tester as ♂, second for tester as ♀.

† R_4R_4 should be rather expected not to pollinate B, D and SP-8; it was found, however, that it can do so—see Discussion.

The class to which the individual progeny plants belonged was determined by the use of certain testers. Table 6 shows the expected results for crosses between the members of all the classes and the testers. For classes A, B, C, and D these reactions had been found by the previous experiments (tables 1 and 4). The designations of the original parents "SP-7" and "SP-8" were taken over for the classes having their respective genotypes—no confusion ever resulted from this practice. The reactions of classes SP-7 and SP-8 were also already known.

Homozygotes R_4R_4, R_3R_3, etc., were expected to occur in the second generation according to the hypothesis. These had not been encountered previously and their reactions had to be deduced. Their expected behavior and the subsequent observations coincided (table 6), with the exception of R_4R_4 which was unexpectedly able to fertilize the types R_1R_4, R_2R_4, R_3R_4 (v. Discussion).

While R_4R_4 had characteristic test reactions of its own, it was not possible by the pollen-tube tests to distinguish R_1R_1 from B and R_3R_3 from class SP-8

TABLE 7

F_2 pollen tube tests. (Experimental results from F_2 cultures.)

CROSS	CULTURE AND PLANT NO.	TESTER PLANTS† B R_1R_4	D R_2R_4	SP-7 R_1R_2	SP-8 R_1R_4	CLASSIFIED	
B×C	106- 7	− −*	+ +		− −	A	
$R_1R_4 \times R_2R_3$	106- 8	− −	+ +	+ −	− −	A	
	106 -2	+ +	−	− −	+ +	D	
	106 -4	+ +	− −	−	+	D	
	106 -5	+ +	− −	− −	+ +	D	
	106 -9	+ +	− −	− −	+ +	D	
	106 -3	− +	− −		+ +	SP-7	
	106 -6	− +	− −	− −	+ +	SP-7	
	106-11	− +	− −	− −	+ +	SP-7	
	106-10	+ +	+ +	+ +	− −	SP-8	Expected: A, D, SP-7, SP-8
	106-12	+ +	+ +		− −	SP-8	Observed: 2A:4D:3SP-7:2SP-8
One plant died prematurely.							
D×A	94- 6	− −	+ +		+ +	B	
$R_2R_4 \times R_1R_3$	94- 4	+ +	− −		− +	C	
	94- 7	+ +	− −		− +	C	
	94- 8	+(−)	−(−)	−	−(−)	C	♂ sterile†
	94-18	+ +	− −		− +	C	
	94- 2	− +	− −		+ +	SP-7	
	94- 9	− +	− −		+ +	SP-7	
	94-10	− +	− −	−	+ +	SP-7	
	94-11	+ +	+ +		− −	SP-8	Expected: B, C, SP-7, SP-8
	94-12	+ +	+ +		− −	SP-8	Observed: 1B:4C:3SP-7:2SP-8
One abnormal dwarf failed to bloom (replaced by -18); two plants died during tests.							
A×C	104- 2	−	+ +	+ −	− −	A	
$R_1R_3 \times R_2R_3$	105 -3	− −	+ +	+ −	− −	A	
	105 -9	− −	+ +	+ −	− −	A	
	104 -1	+ +	− −	− −	− +	C	
	105- 7		− −	− −	− +	C	
	104- 4	− +	− −		+ +	SP-7	
	105 -2	− +	− −		+ +	SP-7	
	105 -5	− +	− −		+ +	SP-7	
	105- 6	− +	− −	− −	+ +	SP-7	
	105 -8	− +	− −	− −	+ +	SP-7	
	105-10	− +	− −	− −	+ +	SP-7	
	104- 3	+(−)	+(−)	+(−)	−(−)	R_3R_3	highly ♂ sterile†
	104 -5	+ +	+ +		− −	R_3R_3	
	104 -6	+ +	+ +	+ +	− −	R_3R_3	104-6, -7, -8 tested with C gave +− as
	104- 7	+ +	+ +		− −	R_3R_3	expected.
	104 -8	+ +	+		− −	R_3R_3	Expected: C, SP-7(A, R_3R_4)
	105-11	+ +	+ +	+ +	− −	R_3R_3	Observed: 2C:6SP-7:3A:6R_3R_3
Two cultures: three plants died prematurely in 105. 104 contained only eight plants.							
D×B	97- 4	− −	+ +	+ −	+ +	B	
$R_2R_4 \times R_1R_4$	97-13	− −	+	+ −	+ +	B	
	97 -2	+ +	− −	− −	+ +	D	
	97- 5	+ +	− −	− −	+ +	D	
	97 -8	+ +	− −	− −	+ +	D	
	97-10	+ +	− −		+ +	D	
	97- 7	− +	− −		+ +	SP-7	
	97-11	− +	− −		+	SP-7	
	97 -1	+ +	+ +	+ +	+ +	R_1R_4	
	97 -3	+ +	+ +	+ +	+ +	R_1R_4	
	97- 6	+ +	+ +	+ +	+ +	R_1R_4	
	97- 9	+ +	+ +	+ +	+ +	R_1R_4	Expected: B, SP-7 (D, R_1R_4)
	97-12	+ +	+ +	+ +	+ +	R_1R_4	Observed: 2B:2SP-7:4D:5R_1R_4

* First sign for tester as ♂; second sign for tester as ♀.

† Male steriles were classified by their stigma reactions alone.

‡ Testers were: for B 74-1, 74-5, 79-4; for D 74-2, 79-2; for SP-7 and SP-8 the original parents and grafts from these.

TABLE 7—(*continued*)

CROSS	CULTURE AND PLANT NO.	TESTER PLANTS† B R_1R_4	D R_1R_4	SP-7 R_1R_2	SP-8 R_2R_4	CLASSIFIED	
A×SP-7	109- 5	− −	+ +		− −	A	
R_1R_2×R_1R_2	109- 6	− −	+ +		− −	A	
	109 -8	− −	+ +		− −	A	
	109-12	− −	+		− −	A	
	109-14	− −	+ +		− −	A	
	110- 4	− −	+ +	+	− −	A	
	110-11		+	−	− −	A	died early
	109- 4	+ +	− −		− +	C	
	109-10	+ +	− −		− +	C	
	110- 1	+	− −		− +	C	
	110-12	+ +	− −	− −	− +	C	
	109 -7	+ +	− −	− −	+ +	D	Fifth, unexpected class
	110- 7	+ +	− −	−	+ +	D	
	110- 8	+ +	− −	− −	+ +	D	
	110-10	+ +	− −	− −	+ +	D	
	109 -3	− +	− −		+ +	SP-7	
	109 -9	− +	− −	− −	+ +	SP-7	
	109-11	− +	− −		+ +	SP-7	
	110- 5	− +	− −		+ +	SP-7	
	109-13	− −	+ +		+ +	R_1R_1	
	110 -2	−	+	−	+ +	R_1R_1	
	110 -3	− −	+ +		+ +	R_1R_1	
	110 -6	− −	+ +	+ −	+ +	R_1R_1	Expected: C, SP-7($A_1R_1R_1$)
	110- 9	− −	+ +	+ −	+ +	R_1R_1	Observed: 7A:4C:4D:4SP-7:5R_1R_1
2 cultures.							
B×SP-7	107 -1	+ +	− −		+ +	D	
R_1R_4×R_1R_2	107 -3	+ +	− −		+ +	D	
	107 -2	− +	− −	− −	+	SP-7	
	107 -7	− +	− −	− −	+ +	SP-7	
	107 -4	−	+ +	+	+ +	B or R_1R_1	
	107 -5	− −	+ +		+ +	B or R_1R_1	
	107 -6	− −	+ +		+ +	B or R_1R_1	
	107 -8	− −	+ +		+ +	B or R_1R_1	
	107 -9	−	+ +		+ +	B or R_1R_1	
	107-10	− −	+ +		+ +	B or R_1R_1	
	107-11	− −	+ +		+ +	B or R_1R_2	Expected: D, SP-7 (B, R_1R_1)
	107-12	− −	+ +		+ +	B or R_1R_1	Observed: 2D:2SP-7:8B or R_1R_1
C×SP-8	98- 1	+ +	− −	− −	− +	C	
R_2R_3×R_1R_3	98- 3	+ +	− −	− −	− +	C	
	99 -2	+(−)	−(−)	−(−)	−(−)	C	♂ sterile†
	100- 1	+ +	− −	− −	−	C	
	98- 4	+ +	− −		+ +	D	
	98- 5	+ +	− −	− −	+ +	D	
	98 -2	+ +	+ +	+	− −	SP-8 or R_1R_3	
	99 -1	+(−)	+(−)	+(−)	−(−)	SP-8 or R_1R_3	♂ sterile†
	99- 3	+ +	+ +		− −	SP-8 or R_1R_3	Expected: D, SP-8 (C, R_1R_3)
	99- 6	died early		+ +		SP-8 or R_1R_3	Observed: 2D:4C:4SP-8 or R_1R_3
Only 10 plants survived from 4 cultures of this cross-incompatible combination.							
SP-8×C	117- 2	+ +	− −	− −	− +	C	
R_1R_4×R_2R_3	117- 6	+ +	− −	− −	− +	C	
	117- 3	+ +	− −	− −	+ +	D	
	117- 4	+ +	− −		+ +	D	
	117- 9	+ +	− −	− −	+ +	D	
	117-10	+ +	− −		+ +	D	
	117 -1	+ +	+ +		− −	SP-8 or R_1R_3	
	117- 5	+ +	+ +	+ +	− −	SP-8 or R_1R_3	
	117 -7	+ +	+ +	+ +	− −	SP-8 or R_1R_3	
	117- 8	+ +	+ +	+ +	− −	SP-8 or R_1R_3	Expected: C, D (SP- 8, R_1R_3)
	117-12	+ +	+	+	− −	SP-8 or R_1R_3	Observed: 2C:4D:5SP-8 or R_1R_3
One plant died prematurely.							

TABLE 7—(*continued*)

CROSS	CULTURE AND PLANT NO.	TESTER PLANTS† B R_1R_2	D R_3R_4	SP-7 R_1R_3	SP-8 R_1R_4	CLASSIFIED	
SP-8×D	116- 2	− −	+ +	+ −	− −	A	unexpected class
$R_1R_4 \times R_3R_4$	116-11	− −	+ +	+ −	− −	A	unexpected class
	116 -3	+ +	− −	− −	− +	C	
	116- 9	+ +	− −	− −	− +	C	
	116- 1	+	+ +	+ +	− −	SP-8	
	116- 5	+ +	+ +	+ +	− −	SP-8	
	116 -8	+(−)	+(−)	+(−)	−(−)	SP-8	♂ sterile†
	116-10	+ +	+ +		− −	SP-8	
	116-12	+ +	+ +		− −	SP-8	
	116- 4	+ +	+ +	+	+ +	R_1R_4	
	116- 6	+ +	+ +		+ +	R_1R_4	Expected: C, D (SP-8, R_1R_4)
	116 -7	+ +	+ +	+	+	R_1R_4	Observed: 2A:2C:0D:5SP-8:3R_1R_4
					Total	120	As expected: 114
							Unexpected: 6

(R_2R_2 was not obtained). To make these distinctions, progeny tests would be necessary which are not included in the present investigation.

Table 7 summarizes the results. The "+" entries were based on a minimum of four stigmata with pollen tubes, regardless of the number of failures; "−" entries, on a minimum of four stigmata without pollen tubes and not a single stigma with tubes. As in table 1, at least three pollen grains on a stigma were necessary for it to be counted and three pollen tubes or more for a positive count (one or two tubes again were exceedingly rare exceptions, either strong or no reactions being the rule). These statements of minima give no real picture of the amount of accuracy obtained as a great number of replications were frequently made, especially in the case of the negative entries, in order to check and countercheck results.

Some classes could be assigned by testing with just one tester; thus, only C gave reciprocally different results with SP-8 and only class SP-7 with tester B. The other heterozygotes could be determined by the use of two testers; e.g., "− −" with B and with SP-8 characterizes members of class A. The use of three or four testers with most individual plants represented an additional check. It can be therefore stated with confidence that misclassifications should have been very uncommon.

The following results were obtained:

Classes B and C which presumably had no allele in common (for genotypes consult table 7 or fig. 1) were expected to produce the progeny classes A, D, SP-7, and SP-8. These four classes were actually observed, with the frequencies 2:4:3:2.

D×A, also with no allele common to the parents gave 1 B:4 C:3 SP-7:2 SP-8 in keeping with the expectation. Plant 94-8 was apparently male sterile. Its pollen which was of normal appearance under the low power of the microscope always failed to produce tubes—even when tested on entirely unrelated plants. This plant was therefore classified on the basis of its stigma reactions.

In the crosses which now follow *the pairs of parents had one factor in common*

and it remained to be seen whether both segregating types of pollen from the male would function in these crosses; that is, whether both R_2 and R_3 pollen or R_2 pollen only would fertilize in a cross $R_1R_3 \times R_2R_3$. In listing the expectations below, the classes expected from pollen carrying the common allele were written in parentheses.

Thus, A×C was expected to yield C, SP-7, (A) and (R_3R_3). Actually observed were 2 C:6 SP-7:3 A:6 R_3R_3. Pollen production in one of the R_3R_3 plants (104-3) was very scant, but the pollen was functional if enough was gathered to cover stigmata of a completely unrelated plant. 104-3 was classified on the basis of its stigma reactions alone.

The cross D×B was expected to give B, SP-7, (D) and (R_4R_4) and these classes were produced in the ratio 2:2:4:5.

The backcross of A to the parent SP-7 with the latter as the male was expected to result in the classes C, SP-7, (A) and (R_1R_1). Culture 110 of this cross was studied first and three plants of the unexpected class D were found. Suspecting a contamination, culture 109, which had originated from the same two parents, was taken from the reserve and its members tested; here again one D plant was found, which made a contamination appear less likely. Thus, in the two cultures together were obtained 7 A:4 C:4 D:4 SP-7:5 R_1R_1, five classes in all instead of the expected four classes. No explanation for this discrepancy can be given.

Progeny classes D, SP-7, (B) and (R_1R_1) were expected to result from B×SP-7. The incompatibility reactions of the latter two are the same and in keeping with the expectation the following were obtained: 2 D, 2 SP-7 and eight plants which could have been either B or R_1R_1.

Pollen-tube tests had indicated that pollen of SP-8 fails on C (tables 1, 4) but when the cross was made and the seed planted each of four cultures of 50 seeds gave a few seedlings (5, 3, 2, and 1 germinants; the numbers of filled achenes were 8+9+3+9=29 or 14.5 percent as entered in table 3). Only ten of these survived to maturity and could be tested for their incompatibility reactions. The expected classes were here D, SP-8, (C) and (R_3R_3), with SP-8 and R_3R_3 indistinguishable, except by progeny test. 2 D, 4 C, and 4 either R_3R_3 or SP-8 were found. Two plants were pollen sterile, as their pollen always failed, even on unrelated plants, though it was of normal appearance under the microscope. These two plants, 99-1 and 99-2, were classified by their stigma reactions.

SP-8×C was expected to give classes C, D, (SP-8) and (R_3R_3) of which the latter two were indistinguishable. Observed were 2 C:4 D:5 SP-8 or R_3R_3.

From SP-8×D were expected C, D, (SP-8) and (R_4R_4); obtained were 2 C, 5 SP-8 and 3 R_4R_4 as expected, but no D plants. In addition, two unexpected A's occurred. No explanation of this deviation from the expectation can be given.

Altogether, a total of 120 plants was tested. Of these, 114 belonged to classes which were expected in their respective cultures and six were unexpected. The most noteworthy result is the occurrence of four classes in all the crosses, with the exception of the unexpected fifth class D in A×SP-7. It is not in contradic-

tion with this statement that only three groups could be observed in those crosses where the homozygous class is indistinguishable from one of the heterozygous ones.

The great ease with which homozygotes were produced in this self-incompatible species was surprising. While the above evidence appears to be irrefutable on this point it is indirect and only the genetic analysis of the presumed homozygotes can bring the final proof. Crosses like $R_3R_3 \times R_4R_4$ should give only one single progeny class and $R_1R_2 \times R_3R_3$ should give two classes.

TABLE 8

*Backcrosses of sibs ex SP-3×SP-6 and SP-6×SP-3 to their parents.**

PLANT NO.	SP-3♀	SP-3♂	SP-6♀	SP-6♂
		Culture 82 (SP-6×SP-3)		
82- 1	+	+	−	−
82- 2	+	−	−	−
82- 4	+	−?		
82- 5	+	+	−	−
82- 7	+	+		
82-10	+	+	−?	−
82-11	+	−	−	−
		Culture 83 (SP-3×SP-6)		
83- 1	+	+	−	−
83- 3	+	−	−	−
83- 4	+	+	−	−
83-11	+	−	−	−
83-13	+	+	−	−
83-16	+	−		
83-17	+	+		
83-22	+	+		

* Criteria used are the same as in table 1.

? One head gave +; probably due to error as retesting gave −.

Families SP-3×SP-6 and SP-6×SP-3

In addition to this analysis of progenies from SP-7 and SP-8, another combination was used; namely, the progenies SP-3×SP-6 and SP-6×SP-3 (table 8). Fifteen plants were tested of the two crosses by pollen-tube tests with their parents and it was found that the pollen from all of these produced tubes in the stigmata of SP-3, but SP-3 pollen failed on six while successful on the remainder. Ten plants of this group were tested with SP-6 and they all proved reciprocally incompatible with this parent.

Existence of a large number of classes

The origin of plants SP-1, SP-2 to SP-15 was described under "Materials." As stated there, it was not known to the author to what extent these plants were interrelated. The results of a survey of this group with respect to their compatibilities is presented in table 9. Tested together with them was a

TABLE 9

*Pollen tube tests for compatibility of 14 plants with no known relationship.**

	SP 1	SP 2	SP 4	SP 7	SP 8	SP 9	SP 10	SP 11	SP 12	SP 13	SP 14	SP 15	IV P	IV F
SP- 1	−	+	+	+		+	+		−		+	+	+	
SP- 2	+	−			+				+	+		+		+
SP- 4	+	+	−	+	+	+		+	+	+		+		+
SP- 7	+			−	+		+		+	+	+	+	+	+
SP- 8	+	+	+	+	−						+			
SP- 9		+	+			−		+	+	+	+		+	+
SP-10		+						+	+	+	+	+		+
SP-11		+	+			+	+				+			+
SP-12	−	+	+		+	+	+	+	−		+	+	+	+
SP-13		+	+		+	+	+	+	+	−			+	+
SP-14	+	+			+	+			+	+	−		+	
SP-15	+		+				+		+		+			
IV-P		+		+	+	+	+			+		+		−
IV-F			+	+			+		+			+	−	−

+ Tubes formed in at least three stigmata.

− Consistently no tubes in five or more stigmata (the two reciprocally incompatible pairs were tested more than 20 times, but for the selfs usually only one head with five stigmata was used).

* Except the mother-daughter pair, IV-P and IV-F.

mother-daughter pair of different origin, plants IV-P and IV-F. As far as tested they were all found to be self-incompatible; a few incompatibilities between plants occurred also. However, the large number of cross-compatibilities is striking; it indicates that there are many incompatibility classes in guayule.

Entries in the table were based on results from one or more heads. Required were at least three stigmata with tubes for "+" and at least five stigmata without any tubes for "−". The few cross-incompatible combinations shown in the table were tested on at least 20 stigmata to eliminate misclassification.

DISCUSSION

Self-incompatibility has been analyzed in many species and the main types of inheritance were brought together and critically reviewed by BRIEGER (1930) and STOUT (1938). There is no reason for repetition. But some comparison of the behavior of guayule with that of some of the more thoroughly analyzed plants will aid in reaching an understanding of the behavior of guayule itself.

The most carefully investigated species is *Nicotiana Sanderae* (EAST and MANGELSDORF 1925). Its self-incompatibility is determined by one series of multiple alleles and any pollen tube carrying an allele which is also present in the style usually fails to reach the ovary. Plants having two alleles in common

are incompatible. Thus, S_mS_n is incompatible with all other S_mS_n plants, besides being self-incompatible. Plants having one common allele like S_mS_n and S_nS_o are compatible, but only one type of pollen grain functions if they are crossed and two classes of progeny result: $S_mS_n \times S_nS_o$ gives S_mS_o and S_nS_o. Four classes result from hybridization of two plants which have no allele in common, as in the cross $S_mS_n \times S_oS_p$. If a homozygote is crossed with a heterozygote reciprocal differences in compatibility result; $S_mS_m \times S_mS_n$ is compatible, because S_n functions in the S_mS_m style, but the reciprocal combination is incompatible as growth of an S_m gametophyte is hindered in a style containing the same allele. Such reciprocal differences, however, are a special case as homozygotes are not usually obtained.

This system has been claimed to apply without further modification to members of very different families; for instance, species of Veronica, Antirrhinum, Nemesia, Oenothera and Trifolium (see the reviews cited above). Some of these have been thoroughly tested and there is no doubt that a species like *Oenothera organensis*, the object of many brilliant studies by S. EMERSON (1938) and D. LEWIS (1947 and other papers) follows this scheme. But there is a tendency to ascribe too general a validity to it and to adopt it for new cases of self-incompatibility without investigating first the details which were proved for Nicotiana. A case in point is *Cosmos bipinnatus* (LITTLE, KANTOR and ROBINSON 1940), which is singled out here because it is a member of the Compositae like guayule. The entire experimental evidence in Cosmos consists in the observation of four incompatibility classes in the second generation which were discovered by means of sib crosses and the authors subsequently argued that the Nicotiana scheme should apply. In guayule there were also four classes found, generally, in the progeny of any two plants, excepting homozygotes, but the deviations from the Nicotiana scheme are obvious. These are: (1) The frequent failure of guayule offspring to be compatible with their mother, while in Nicotiana only rare homozygotes are incompatible with their mother, and then only as pollen parents. This mother-offspring incompatibility and incompatibilities between certain sib classes in guayule, as between A and B, show that in guayule plants which have one allele in common frequently fail to cross; this will be discussed in more detail below. In Nicotiana one-half of the pollen from a plant having an allele not present in the mate is functional, even if the second allele is present in both. (2) The ease with which homozygotes can be obtained in guayule whereas in Nicotiana they occur only by special manipulation; that is, by artificial pollination of closed buds or at the end of the season. If homozygotes were easily obtainable in Nicotiana the system would break down. (3) The number of classes produced in crosses between heterozygotes in guayule is generally four, whereas in Nicotiana either four or two may result, depending on whether the parents had one or no allele in common.

The behavior of *Capsella grandiflora* is of a different nature (RILEY 1932, 1936). RILEY finds only three inter-sterile classes in his material and no more. Crosses may result in one, two or all three of these classes. Reciprocal crosses are either both compatible or both incompatible and reciprocal differences

are not found. To explain these results RILEY postulates the existence of two pairs of unlinked factors which control incompatibility, with one allele epistatic over the others.

The mechanism in guayule is obviously different, with four to six classes (including the parents) found in every family and the frequent occurrence of differences in reciprocal crosses.

But the two species have important features in common. In Capsella, the genotype of a sporophyte determines whether its pollen will be compatible or incompatible on a given pistil, not as in Nicotiana, where the reaction of a pollen grain is determined in the gametophyte itself. In the latter, in a cross $S_mS_n \times S_nS_o$ pollen grains containing S_n and S_o behave independently and only S_o pollen functions in fertilization. In Capsella, all grains from one plant act alike: in the cross *Ttss*×*Ttss* both *Ts* and *ts* grains are inhibited by the presence of dominant *T*. The *ts* grains from a plant *ttss*, on the other hand, do germinate on a *Ttss* style, but not on a *ttss* style. This example from Capsella illustrates the sporophytic determination and the occurrence of dominance, two phenomena also found in guayule, as will be seen below. It should be noted here that dominance, in Capsella, occurs in both the male and the female components.

Other examples of sporophytic control of self-incompatibility are the heterostylic plants (that is, those where the sexual organs differ in length in different individuals; see reviews by LEHMANN 1928, and BRIEGER 1930). One pair of alleles (or several closely linked alleles, ERNST 1928) controls self-incompatibility and floral characteristics in the distylic primroses and at least two pairs are effective in tristylic plants like *Lythrum salicaria*. Also *Cardamine pratensis*, which is homostylic, exhibits sporophytic control of self-incompatibility and CORRENS (1912) postulated one series of four alleles for this species.[3]

It is characteristic of all these species with sporophytic control of incompatabilities that differences between reciprocal crosses do not occur; if two plants are prevented from crossing by the presence of a certain allele in both,

[3] The behavior of *Cardamine pratensis* has caused much bewilderment. CORRENS (1912) crossed ♀ and ♂ and grouped the progeny into four classes: a) compatible with both ♀ and ♂, b) incompatible with ♀, compatible with ♂, c) compatible with ♀, incompatible with ♂, d) incompatible with ♀ and ♂. He assigned the genotype *BG*, *Bg*, *bG* and *bg* to them and formulated the following hypothesis: 1) *B*, *b*, *G* and *g* are alleles, 2) *B* and *G* are active, *b* and *g* inactive, 3) pollen-tube control is sporophytic. It is unfortunate that CORRENS designated *b* and *g* as inactive and called them "recessive" only parenthetically in quotation marks (p. 203). His stressing of the presumed inactivity of *b* and *g* created the main reason for all the perplexities caused by the hypothesis to its author as well as to many others, who subsequently forgot all about CORRENS' mention of recessivity. If CORRENS had designated *B* and *G* as dominant over *b* and *g* (with dominance effective in both pistil and pollen, see text) and the latter recessive (but active) all this would have been avoided. The relations of the four classes to their parents and to each other fall naturally into such a scheme, without the necessity for building a supportive hypothesis to explain the self-incompatibility of the "doubly inactive" genotype *bg*.

This simple hypothesis fits the data of CORRENS. LAWRENCE (1930) and BEATUS (1934), the latter employing plant material of his own, attempted to explain the data with more elaborate schemes involving several series of alleles—*C. pratensis* is a tetraploid—and genotypic control of incompatibility.

it is of no concern which plant is used as the female and which as the male. It is evident, then, that guayule does not follow these schemes, at least not without modification.

Reciprocal differences in compatibility have been described in a number of instances. Already cited was the case of homozygotes in Nicotiana. In guayule the situation is not that simple as here plants which crossed in one direction but failed in the other gave four classes in their compatible crosses, as for instance the classes A and C; the cross C×A was incompatible, while the reciprocal A×C gave four classes as gave progenies from D×A, and B×C. Thus, neither A nor C can be a homozygote for a single determinant of self-incompatibility.

SIRKS (1926, 1927) modified the Nicotiana scheme to explain the reciprocal differences which he found in *Verbascum phoeniceum*. He postulated S_1 to be incompatible in styles containing S_2 as well as in those with S_1 and, similarly, S_3 pollen to fail on both S_3 and S_4 styles. Here, as in Nicotiana, only two classes should be formed in progenies from plants having a common factor, and SIRKS' modification, therefore, cannot explain the guayule case. Incidentally, an interesting corollary of the Verbascum scheme is the failure of the cross $S_2S_4 \times S_1S_3$ of two genotypes which have no common allele.

The occurrence of reciprocal differences in *Brassica oleracea* has been explained by KAKIZAKI (1930) by assuming an interaction between two pairs of multiple alleles, of which one interferes with and the other promotes pollen-tube growth. LAWRENCE (1930) shows that reciprocal differences in some cases, including *Verbascum phoeniceum* could be due to the interaction of two series of incompatibility alleles brought together by allopolyploidy. However, the hypotheses of these authors are not applicable to guayule, as the classes in the progenies would very likely number more than four.

The preceding discussion emphasizes the following points:

1) Self-incompatibility in guayule is controlled by a series of multiple alleles, as in Nicotiana, and a single such series is sufficient to explain the results which were obtained. Figure 1 is a hexagon, the corners of which designate the six classes of the family SP-7×SP-8 (and its reciprocal). The three types of connecting lines between the corners indicate the compatibilities and incompatibilities which were observed as explained in the legend. Below the class names are entered the Mendelian symbols adopted for each class: R_1R_2 for SP-7, R_3R_4 for SP-8 and their four recombinations for the four progeny classes.

2) The incompatibility reaction takes place between two sporophytes, as in Capsella. It is not necessary to postulate with RILEY (1936, p. 37) that the incompatibility substance of the pollen is produced during the reduction division of the pollen mother cell, as we have no exact knowledge of the when and the where. The reaction is one between cytoplasm produced in the male organ and a pistil. One cannot assume, however, that the same incompatibility substance, or substances, are diffused through the protoplasm of the entire plant, since an incompatibility *reaction* could then not take place; there must be two different substances which are able to react with each other, syn-

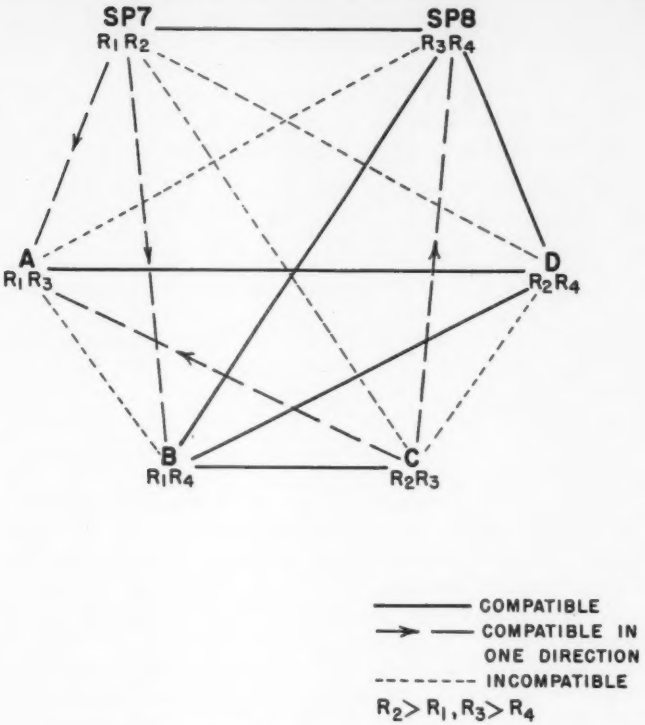


FIGURE 1. Cross-compatibilities and incompatibiiities in one family of diploid guayule.

thesized at two different places, the androecium and the gynoecium, by the same gene (or, as LEWIS (1949) recently postulated, by two self-reproducing parts between which no crossing over takes place).

3) The reciprocal differences which occur in the guayule material may be explained by assuming that certain alleles are dominant over certain others in the male, but not in the female. Thus, R_2 is dominant over the remainder, as reciprocal differences (see fig. 1) were obtained in the following crosses, with the arrow indicating the only way pollinations could be made:

$R_1\underline{R_2}$ (SP-7)	$R_1\underline{R_2}$ (SP-7)	$\underline{R_2}R_3$ (C)	$\underline{R_2}R_3$ (C)
↓	↓	↓	↓
R_1R_3 (A)	R_1R_4 (B)	R_1R_3 (A)	R_3R_4 (SP-8)

In each of these crosses R_2 was present in the male parent, but absent from the female. In addition the pairs had one factor in common; its ineffectiveness in association with R_2 in the pollen parent indicates dominance of R_2. In the

reciprocal crosses where R_2 was present in the style, the alleles associated with it prevented pollen-tube growth which shows that there is no dominance in the style.

Those crosses in which R_2 was present in both mates could not be made, regardless of which factor was associated with it; this also points towards dominance of R_2 over the other three factors:

$R_1\underline{R_2}$ (SP-7)	$R_1\underline{R_2}$ (SP-7)	$\underline{R_2}R_3$ (C)
⋮	⋮	⋮
$\underline{R_2}R_3$ (C)	$\underline{R_2}R_4$ (D)	$\underline{R_2}R_4$ (D)

The factor R_4 is recessive to the other three as its presence in two heterozygotes causes no incompatibilities between them—the crosses

$R_3\underline{R_4}$ (SP-8)	$R_3\underline{R_4}$ (SP-8)	$R_2\underline{R_4}$ (D)
↕	↕	↕
$R_1\underline{R_4}$ (B)	$R_2\underline{R_4}$ (D)	$R_1\underline{R_4}$ (B)

were all compatible in both directions. (R_4, however, is not a self-fertility factor as homozygotes R_4R_4 were self-incompatible.)

R_1 and R_3 have equal dominance values as the following reciprocally incompatible crosses show; they indicate that either factor of a pollen parent of class A (R_1R_3) is equally effective in preventing fertilization if opposed by the same factor:

$\underline{R_1}R_3$ (A)	$R_1\underline{R_3}$ (A)
⋮	⋮
$\underline{R_1}R_4$ (B)	$\underline{R_3}R_4$ (SP-8)

As noted above, in Capsella dominance is exhibited in both the pollen and the female organ. Also in artificial tetraploids of *Oenothera organensis* dominance of certain alleles over others occurs in the diploid pollen grains but not in the styles (LEWIS 1947). LEWIS interprets this as indicating that in the past history of the species modifiers have been selected to suppress dominance in the stylar tissue as interfering with a full expression of incompatibilities. Diploid pollen has not played a role before and where it is produced artificially dominance as a primary property of certain alleles is made evident. In guayule the absence of dominance in the stigma and its presence in the pollen could hardly have arisen in this way, as presumably diploid control was present in both from the beginning.

4) In Nicotiana two classes are obtained where mates have one factor in common. For the guayule case it is postulated that all pollen grains of a plant act alike; as a consequence four classes should arise in all crosses between heterozygotes even if they carry one common factor. This actually was found to be true, in general, as shown in table 7.

If the parents have no common allele the parental classes are not recovered in the progency, but only the recombinations. If the parents have a common allele the following progeny classes resulted in each case: The paternal class, a new class (these two classes are also recovered in Nicotiana from a cross of the type $S_1S_2 \times S_1S_3$); third, the maternal class and fourth, a homozygous class. The following example from the data (culture 97) may be cited as an illustration:

$$R_2R_4 \text{ (D)} \times R_1R_4 \text{ (B)} \rightarrow R_1R_4 \text{ (B, paternal)} + R_1R_2 \text{ (SP-7, new)} + R_2R_4 \text{ (D, maternal)} + R_4R_4.$$

In similar fashion the homozygotes R_1R_1 and R_3R_3 could be recovered. The production of R_2R_2 could not be accomplished because no two plants carrying dominant R_2 could be crossed.

The homozygotes of R_1 and R_3 obtained in the various progenies behaved as could be expected of them (tables 6 and 7) giving reciprocal differences where crossed with plants carrying R_2 in addition to R_1 or R_3, respectively, and no reciprocal differences where R_2 was absent. The homozygotes for recessive R_4, however, gave no reciprocal differences in any case. R_4 apparently caused incompatibility only if present in homozygous form in both mates: R_4R_4 was self-incompatible.

Guayule is not unique in possessing this syndrome of one series of incompatibility alleles, sporophytic control of the incompatibility reactions and dominance of certain alleles over others in the pollen. The priority for having recognized this mechanism of self-incompatibility in any species belongs to DR. MORRIS B. HUGHES who worked with *Crepis foetida* subsp. *rhoeadifolia*, another member of the Compositae. As the Ph.D. thesis (1943) in which HUGHES presented his material has not yet been published and is therefore not readily accessible, his results and conclusions are summarized below.

HUGHES crossed two plants which were designated later as belonging to classes V and VI and obtained four progeny classes I, II, III and IV. Figure 2 gives the cross relations between these six classes together with the Mendelian symbols assigned to each class. It can be seen there that in Crepis, as in guayule, certain inter-class incompatibilities occurred and reciprocal differences were observed also.

To explain his results, HUGHES made the following postulates (not quoted verbatim).

1) Four alleles are responsible for the incompatibilities found in the families investigated.

2) Pollen behavior is sporophytically determined; that is, the parental genotype is impressed upon the cytoplasm of all the pollen grains from any one plant.

3) S_3 and S_4 are dominant over S_1 and S_2 but not dominant over each other. S_2 is dominant over S_1. The ultimate recessive of the series, S_1, prevents fertilization only when homozygous in both the male and the female involved. Therefore, compatible crosses between two heterozygous plants carrying it will give some S_1S_1 homozygotes.

The similarity of the behavior of this Crepis material with guayule is striking. S_3 and S_4 behave like R_2 of guayule; their homozygotes cannot be obtained because no two plants containing the same dominant allele can be crossed. S_2 in Crepis corresponds to R_1 and R_3 in guayule. HUGHES probably obtained S_2S_2 homozygotes in his cross $S_1S_2 \times S_2S_4$; but without progeny test he was unable to distinguish S_2S_2 from S_1S_2—just as in guayule the homo-

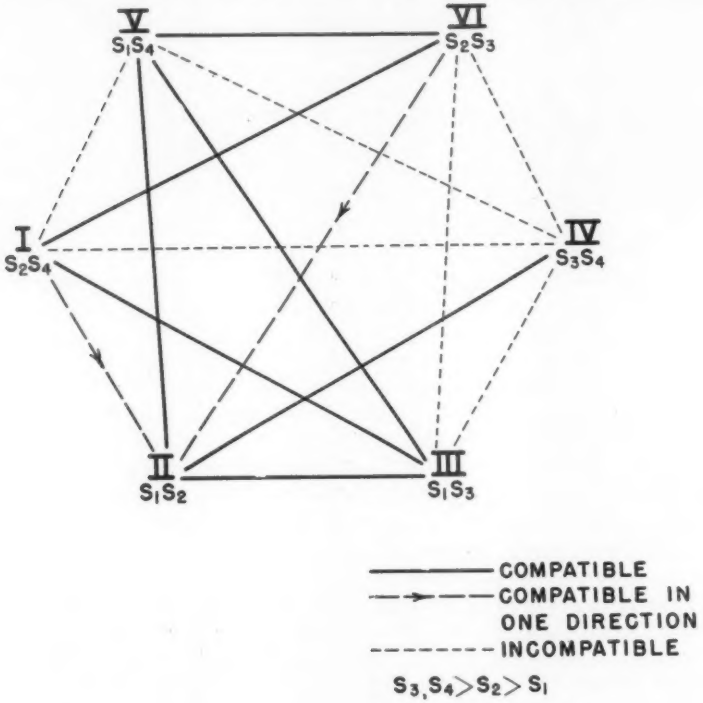


FIGURE 2. Cross-compatibilities and incompatibilities in *Crepis foetida* subsp. *rhoeadifolia* (based on HUGHES 1943)

zygotes of the intermediate alleles R_1 and R_3 exhibited the incompatibility behavior of certain heterozygotes. Finally, S_1 and R_4, the ultimate recessives in the Crepis and guayule series prevented only the selfing of homozygotes but were ineffective in any crosses between homozygotes and heterozygotes.

Crepis foetida subsp. *rhoeadifolia* and guayule are the only two members of the Compositae in which the heredity of incompatibilities has been sufficiently analyzed, as far as is known to the author. Reports on a few other Compositae can be found in the literature but the results do not warrant definite conclusions. One such case is *Cichorium Intybus* where STOUT (1916,

1917, 1918) described great irregularity of behavior. The case of *Cosmos bipinnatus* (LITTLE, KANTOR, and ROBINSON 1940) was mentioned above. LAWRENCE (1930) has made some observations on Dahlia which are of great interest here. To quote from his paper: "Partial compatibility and the 'one-way' compatibility are common phenomena in this hexaploid Prunus, and even more common in the octoploid Dahlia (*D. variabilis*). In this last species several cases are known of seedlings failing with their female parent but setting with their male. This has not been found in the plum. The evidence strongly suggests that until relatively homozygous forms are raised by selection and inbreeding this phenomenon of seedlings failing on their female parents is characteristic of polypoids. No such case is known within a diploid species." Two such cases, found since, are described here and the question arises whether the failure of the pollen of some Dahlia seedlings on their mothers may be the consequence of the "Composite type" of incompatibility system, rather than of polyploidy. (*Crepis foetida* with $2n=10$ chromosomes undoubtedly is a diploid. The elevated chromosome number of 36 in guayule may be suggestive of polyploidy; also DR. ROLLINS has informed me that $2n=24$ occurs in *P. bipinnatifidum*, in another section of the genus. The inheritance of incompatibility described here certainly is that of a diploid or "diploidized" plant.)

Only the analysis of additional species of the Composite family can decide whether there is a distinctive "Composite type" of incompatibility as suggested here and the field is large; EAST (1940) has estimated the number of self-incompatible species in this family at several thousand.

For a discussion of self-incompatibility in the tetraploid guayule material, of which the colchicine produced generation was studied, the reader is referred to the previous paper in this series (GERSTEL and RINER 1950).

Results of the nature presented in table 8 could obviously be obtained where two strongly dominant alleles are present in one parent (SP-6) and an intermediate and the ultimate recessive in the other parent (SP-3). These data indicate that in certain crosses all of the progeny may be incompatible with one parent; such a situation would impose an additional handicap upon the guayule breeder, who has to depend in his work upon the use of backcrosses, at least in the solution of some of his problems. Self-incompatibility always interferes with the use of inbreeding methods, but guayule is even more difficult to manipulate than plants having the Nicotiana-type of incompatibilities, because of greater restrictions upon the use of sib crosses and the frequent failure of backcrosses.

Finally, the data in table 9 on the compatibilities between fourteen plants, presumably not all related to each other, are included as they demonstrate that the number of different incompatibility alleles in existence is considerable.

SUMMARY

A progeny of two diploid guayule plants was found to consist of four different incompatibility classes. All crosses within these classes were incompatible, as were crosses between certain classes and between some classes and the

parents. Reciprocally different results were obtained in crosses between two sib classes and in some parent progeny crosses.

These results were based on pollen-tube tests, but seed-setting tests made on a modest scale gave similar results indicating that no incompatibility barrier existed in this material independently from the one located in the stigmata. In only one case an appreciable amount of seed was obtained where none was expected.

Reciprocal crosses between the two parents gave the same four progeny classes. Tetraploid progeny plants, produced by colchicine treatment, reacted the same as their diploid sibs in selfing and in crossing tests.

It was consequently postulated that there exists one series of multiple alleles, which were named R_1, R_2, R_3, and R_4 in distinction from the S alleles of the Nicotiana type of self-incompatibility. Incompatibilities were assumed to be sporophytically controlled; that is all the pollen grains from one plant act alike and depend on both alleles present in the pollen parent. Dominance in the pollen, but not in the stigma of R_2 over the other alleles and of R_1 and R_3 over R_4 would explain the reciprocal differences obtained in certain crosses.

Nine second generation cultures containing in all 120 plants were produced in part by backcrossing and in part from sib crosses. They bore out, in general, the expectations based on the working hypothesis with the exception of six plants of unexpected genotypes which had appeared in two of the cultures. Five pollen sterile plants had to be classified on the basis of their stigma reactions.

In keeping with the hypothesis four progeny classes were obtained even where two heterozygous parents had one allele in common and plants homozygous for self-incompatibility alleles occurred in abundance.

In an unrelated family, parent-progeny tests indicated that the principles of inheritance and control were probably the same, with a different distribution of dominant and recessive alleles. Here one parent was incompatible in all crosses with all of its offspring which situation must present a particular handicap upon plant breeding procedures.

The existence of a large number of alleles in the species was suggested by the occurrence of few cross-incompatibilities among a sample of 14 plants of which only two were known to be closely related.

Thus, the type of self-incompatibility found in guayule combines a feature from Nicotiana; namely, the extended series of multiple alleles, and another from Capsella; namely, sporophytic control with a third which is known from tetraploid *Oenthera organensis;* namely, dominance expressed in the pollen, but not in the style. This type has been found to occur in only one other plant, a species of Crepis, which, like guayule, is a member of the Compositae.

ACKNOWLEDGMENTS

The writer is deeply indebted to Miss CAROL KIDD for diligently performing a large proportion of the tedious pollen-tube tests and to DR. R. C. ROLLINS for placing the parent plants at his disposal. DR. H. M. TYSDAL stimulated

the work by many a vivid discussion of the research and of the manuscript. Professor F. G. BRIEGER suggested designating the incompatibility locus by a new letter in order to point out the difference from the *S* scheme of Nicotiana. The author also wishes to thank DR. G. W. BEADLE whose interest made possible the continuation of the work.

LITERATURE CITED

BEATUS, R., 1934 Die Selbststerilität von *Cardamine pratensis*. Jb. wiss. Bot. **80:** 457–503.

BENEDICT, H. M., and JEANETTE ROBINSON, 1946 Studies on the germination of guayule seed. U. S. Dep. Agric. Tech. Bull. **921:** 48 pp.

BRIEGER, F., 1930 Selbststerilität and Kreuzungssterilität im Pflanzenreich und im Tierreich. xi+395 pp. Berlin: Julius Springer.

CLAUSEN, J., D. D. KECK, and W. M. HIESEY, 1945 Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. Carnegie Inst. Wash. Publ. **546:** vii+174 pp.

CORRENS, C., 1912 Selbststerilität und Individualstoffe. Festschr. med.- naturwiss. Ges., **84.** Vers. dtsch. Naturforsch. u. Ärzte, Münster (Westf.): 186–217.

EAST, E. M., 1940 The distribution of self-sterility in the flowering plants. Proc. Amer. Phil. Soc. **82:** 449–518.

EAST, E., and A. J. MANGELSDORF, 1925 A new interpretation of the hereditary behavior of self-sterile plants. Proc. nat. Acad. Sci. **11:** 166–171.

EMERSON, S., 1938 The genetics of self-incompatibility in *Oenothera organensis*. Genetics **23:** 190–202.

ERNST, A., 1928 Zur Vererbung der morphologischen Heterostyliemerkmale. Ber. dtsch. bot. Ges. **46:** 573–588.

1944 Intra- und interspezifische Bestäubungen an dimorphen Primula-Arten der Sektion Candelabra und ihre Aufschlüsse zum Heterostylieproblem. Arch. Klaus-Stift. **19:** 265–311.

FAGERLIND, F., 1937 Embryologische, zytologische und bestäubungsexperimentelle Studien in der Familie Rubiaceae nebst Bemerkungen über einige Polyploiditätsprobleme. Acta Hort. berg. **11:** 195–470.

GERSTEL, D. U., 1947 Genetics of self-incompatibility. Final Rep., Nat. Rubber Res. Proj., Stanford Res. Inst., Mimeographed.

GERSTEL, D. U. and MARY E. RINER, 1950 Self-incompatibility studies in guayule. I. Pollen-tube behavior. J. Hered. **41:** 49–55.

GUSTAFSSON, Å., 1937 Över förekomsten av en sexuell population inom *Taraxacum vulgare*-gruppen. Bot. Notiser: 323–336.

HUGHES, M. B., 1943 Self-sterility in *Crepis foetida* L. subsp. *rhoeadifolia* (Bieb.) Schinz et Keller. Thesis, filed in the Library, University of California (Berkeley). 86 pp.

KAKIZAKI, Y., 1930 Studies on the genetics and physiology of self- and cross-incompatibility in the common cabbage (*Brassica oleracea* L. var. *capitata* L.). Jap. J. Bot. **5:** 133–208.

LAWRENCE, W. J. C., 1930 Incompatibility in polyploids. Genetica **12:** 269–296.

LEHMANN, E., 1928 Selbststerilität, Heterostylie. Handb. Vererbgswiss. Bd. II. 43 pp. Berlin: Gebr. Borntträger.

LEWIS, D., 1947 Competition and dominance of incompatibility alleles in diploid pollen. Heredity **1:** 85–108.

1949 Incompatibility in flowering plants. Biol. Rev. **24:** 472–496.

LITTLE, T. M., J. H. KANTOR, and B. A. ROBINSON, JR., 1940 Incompatibility studies in *Cosmos bipinnatus*. Genetics **25:** 150–156.

NYGREN, A., 1946 The genesis of some Scandinavian species of Calamagrostis. Hereditas **32:** 131–262.

RILEY, H. P., 1932 Self-sterility in Shepherd's Purse. Genetics **17:** 231–295.

1936 The genetics and physiology of self-sterility in the genus Capsella. Genetics **21:** 24–39.

SIRKS, M. J., 1926 Further data on self- and cross-incompatibility of *Verbascum phoeniceum*. Genetica **8**: 345–367.

1927 The genotypical problems of self- and cross-incompatibility. Mem. hort. Soc. N. Y. **3**: 325–343.

STOUT, A. B., 1916 Self- and cross-pollinations in *Cichorium Intybus* with reference to sterility. Mem. N. Y. bot. Gdn. **6**: 333–454.

1917 Fertility in *Cichorium Intybus*. The sporadic occurrence of self-fertile plants among the progeny of self-sterile plants. Amer. J. Bot. **4**: 375–395.

1918 Fertility in *Cichorium Intybus*. Self-compatibility and self-incompatibility among the off-spring of self-fertile lines of descent. J. Genet. **7**: 71–103.

1938 The genetics of incompatibilities in homomorphic flowering plants. Bot. Rev. **4**: 275–369.

ZĀMELIS, A., and A. MELDERIS, 1932 Pseudogamie bei der selbststerilen *Veronica pinnata* L. infolge der Bestäubung mit dem Pollen von *Veronica longifolia* L. Acta Hort. bot. Univ. latv. **6**: 159–191.

McGraw-Hill

PRINCIPLES OF GENETICS

by E. W. SINNOTT
Sterling Professor of Botany and Director
Sheffield Scientific School, Yale University

LESLIE C. DUNN
Professor of Zoology, Columbia University

and THEODOSIUS DOBZHANSKY
Professor of Zoology, Columbia University

New Fourth Edition

In press

The new edition of this authoritative and well-known college text has been entirely rewritten, incorporating the many additions to the field of genetics which have occurred within the last ten years. The authors have preserved its essential character as a general introduction to the principles and actual problems of genetics. New sections cover such topics as population genetics and speciation, mechanisms of genic effects on development, general principles of population genetics based on the Hardy-Weinberg equilibrium, and the use of fungi and microorganisms in the study of genetics.

Send for a copy on approval

McGRAW-HILL BOOK COMPANY, Inc.
330 WEST 42ND STREET NEW YORK 18. N.Y.

Seeds for Albino Seedlings, etc.

(to show simple Mendelian ratios)

Sorghum, for growing green and albino seedlings
3:1 segregation (100 seeds per packet) $1.00

Sorghum, for growing red and green seedlings,
3:1 segregation (100 seeds per packet) 1.00

Sorghum, parental strains, red and green,
(25 seeds of each) .50

Please make checks payable to the Brooklyn Botanic Garden, Brooklyn, N.Y.

LA CELLULE

Recueil de Travaux originaux de Cytologie, Biologie et Histologie générale. Fondé par J. B. Carnoy en 1884. Publié par P. Debaisieux et P. Martens, Louvain (Belgique).

Trois fascicules constituent un tome, généralement annuel, de 400 à 450 pages in quarto, avec nombreuses planches hors-texte et des figures de texte. Prix de souscription: Frs belges 550, port inclus. Adresser les souscriptions à l'éditeur: Librairie Universitaire, rue de la Monnaie, Louvain. Pour toute autre communication s'adresser au Prof. P. Martens, rue Marie-Thérèse, 23 Louvain (Belgique).

JOURNAL OF GENETICS

Editor: J. B. S. HALDANE

FOUNDED in 1910 the journal publishes records of original research in Heredity Variation and allied subjects, and also from time to time articles summarizing the existing state of knowledge in the various branches of Genetics. All articles are illustrated both with text-figures, and plates, the latter in black and white, and colors.

Subscription price, per volume of 3 parts is 50s, net post free. Single parts 20s net, postage extra. Quotations for back numbers on application.

Orders to

CAMBRIDGE UNIVERSITY PRESS
LONDON: Bentley House, N.W.1.
NEW YORK: 51 Madison Ave.

ECOLOGICAL MONOGRAPHS

(Official Publication of the Ecological Society of America)

First issue published December, 1930

Managing Editors: A. S. PEARSE and H. J. OOSTING, *Duke University*

A quarterly journal devoted to the publication of original researches of ecological interest from the entire field of biological science. The journal will work in close cooperation with *Ecology* and will undertake the publication of papers of from 25 to 100 printed pages in length while *Ecology* will continue to specialize on papers of about 20 printed pages or less.

Published in January, April, July, and October

SUBSCRIPTION: $6.00

DUKE UNIVERSITY PRESS
COLLEGE STATION, BOX 6697
DURHAM, NORTH CAROLINA

An inexpensive and practical method of keeping informed of current biological and nutritional literature.

ADVANCE ABSTRACT CARD SERVICE

Issued promptly and in advance of publication of scientific papers appearing in Journal of Morphology, The Journal of Experimental Zoology, The Journal of Nutrition, The Journal of Comparative Neurology, The American Journal of Anatomy, Journal of Cellular and Comparative Physiology, The Anatomical Record, American Journal of Physical Anthropology, Supplemental Issues, and The American Anatomical Memoirs.

Annual Subscription—$2.50

Send order with remittance to

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
36th Street and Woodland Avenue
Philadelphia 4, Pa.

BIOLOGICAL ABSTRACTS

Covers the world's biological literature

How do you keep abreast of the literature in your field? Perhaps some relatively obscure journal has published a revealing paper on the very subject in which you are most interested. Informative, concise abridgments of all the significant contributions will be found in *Biological Abstracts*.

As well as the complete edition, covering all fields of biology, *Biological Abstracts* also is published in nine low-priced sectional editions which are specially designed for individuals who are interested only in one or more closely related fields. Section A affords a very complete coverage of the Genetics literature—and it is priced at only $5.00 a year (Foreign $5.50).

Write for a sample copy.

BIOLOGICAL ABSTRACTS
University of Pennsylvania
Philadelphia 4, Pa.

Stain Technology

A Journal for Microtechnic

The latest developments in staining and general microscopy are presented in two different ways:

Original articles, 30-40 each year.

Abstracts of articles appearing elsewhere, so worded that the technic may be followed without consulting the original paper.

Issued quarterly **$4.50 a year**
20-year index **$1.50**

Published 1946

5th Edition

Biological Stains

By H. J. Conn, Biological Stain Commission

Cloth, 5½ by 8¼ in.
346 pages

Price $4.25
(If cash accompanies order: $3.75)

For sale by
BIOTECH PUBLICATIONS
Lock Box 269, Geneva, N.Y.

ANNALS OF EUGENICS

A JOURNAL OF HUMAN GENETICS

Edited by L. S. PENROSE, assisted by JULIA BELL, R. A. FISHER, J. B. HALDANE, MARY N. KARN, R. R. RACE, J. A. FRASER ROBERTS

Annals of Eugenics, XV.3 June 1950

Rhesus type D^u. P. H. Renton and F. Stratton.
Measurements of infants at birth. A. Low.
Congenital total colour-blindness associated with oto-sclerosis. A. G. MacGregor and R. Harrison.
The combination of blood groups in a sample of 475 people in a London hospital. D. Bertinshaw, S. Lawler, H. Holt, B. Kirman and R. R. Race.
Data for the study of linkage in man: red hair and the ABO locus. L. S. Penrose.
Taste deficiency for phenylthiourea in African negroes and Chinese. N. A. Barnicot.
Inheritance of the Lutheran blood groups in 47 English families. S. Lawler.
Inheritance of Rh blood groups. D. Bertinshaw, S. Lawler, R. Race, R. Sanger.
Blood group frequencies in a mother and infant sample of the Australian population. L. Bryce, R. Jakobowics, N. McArthur and L. S. Penrose.
Note. Factorial experiments. D. Finney.
Reviews.

Subscriptions, in advance, 50s. per volume.
Four parts, obtainable separately at 15s. each.
Back volumes 55s. per volume.

Orders should be placed with
UNIVERSITY PRESS
CAMBRIDGE, ENGLAND

AMINO ACIDS AND PROTEINS

Cold Spring Harbor Symposia on Quantitative Biology

Volume XIV (1949), 232 quarto pages, 10 plates and numerous figures

Authoritative reviews of many aspects of recent investigations of amino acids and proteins, presented in 22 papers and edited discussions. Subjects considered are fractionation of proteins, protein hydrolysis, amino acid analysis, structure of proteins, metabolism and synthesis of amino acids and proteins. Table of contents sent on request.

Previous volumes still available: XI (1946) Heredity and Variation in Microorganisms; XII (1947) Nucleic Acids and Nucleoproteins; XIII (1948) Biological Applications of Tracer Elements.

Price per volume: $7.00 (postage extra; domestic 25¢, foreign 50¢).

Address: Biological Laboratory
Cold Spring Harbor, New York

NEW PUBLICATIONS RECIEVED

Hutt, F. B. Genetics of the Fowl. 1st. ed. N.Y., McGraw-Hill Book Company, Inc., 1949. xii, 590p. illus., maps, ports. O. $6.50.

Huxley, Julian. Heredity, East and West. Lysenko and world science. N.Y., Henry Schuman, Inc., 1949. x, 246p. portrait. O. $3.00.

Sears, Paul B. Charles Darwin; the naturalist as a cultural force. N.Y., Charles Scribner's Sons, 1950. xii, 124p. O. (Twentieth Century Library) $2.00

Loma, Jose Luis de la, Ideas nuevas e ideas viejas sobre la evolucion y la herencia. Mexico, Chapingo, Edo de Mexico, 1949. 44p. illus. O.

Amino Acids and Proteins, Cold Spring Harbor Symposia on Quantitative Biology, vol. 14, 217, xii pages, quarto, 105 figures (text and 11 plates), published by the Biological Laboratory, Cold Spring Harbor, New York. Price $7.00. 1950.

Lwoff, Andre. Problems of morphogenesis in ciliates; the kinetosomes in development, reproduction and evolution. N.Y. John Wiley & Sons, Inc., London, Chapman & Hall Ltd., 1950. x, 103p. 32 fig. O. Price $2.50.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, or in any scientific field, if of such a character as to be of primary genetic interest.

The length of manuscripts will be limited to twenty-five printed pages (about twelve thousand words) except by special vote of the Editorial Board. In case unusually extensive tabular matter cannot be printed, it will be kept on file on request, provided two copies are furnished by the author.

The *Galton and Mendel Memorial Fund* established in 1923 from donations of biologists and other persons interested in the progress of biological discovery is available to cover the cost of reproducing colored and halftone plates and of printing expensive tables.

Authors should prepare their manuscripts so as to conform to the general usage in GENETICS, particularly in regard to references to literature, arrangement of "Literature Cited" and inclusion of a summary. In case of "Acknowledgements," these should follow the "Summary." Footnotes should be avoided in the text whenever possible and their content be inserted, in parentheses, after the appropriate sentence. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in tables. Legends for figures and plates should be typewritten separately from the illustrations. In addition to the original illustrations photographic copies should accompany the manuscript. Tables also should be type-written separately. The metric system should be used whenever possible.

Galley proofs and page proofs will be sent to authors, except in case of authors resident overseas who, in general, will receive galley proofs only. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript.

GENETICS furnishes 50 reprints, without covers, free. Covers and additional reprints may be secured at cost.

Manuscripts and editorial correspondence should be addressed to the Editor of GENETICS, Department of Zoology, University of California, Berkeley 4, Calif.

GENETICS Vol. 35 No. 4 JULY, 1950

CONTENTS OF PRECEDING ISSUES

NOVEMBER, 1949

ANDERSON, E. G., A. E. LONGLEY, C. H. LI, AND K. L. RETHERFORD, Hereditary effects produced in maize by radiations from the Bikini atomic bomb I. Studies on seedlings and pollen of the exposed generation.

FOX, ALLEN S., Immunogenetic studies of *Drosophila melanogaster* II. Interaction between the *rb* and *v* loci in production of antigens.

GEIRINGER, HILDA, Chromatid segregation of tetraploids and hexaploids.

HECHT, ADOLPH, Tetraploid ratios of self-sterility alleles: a correction.

HANSON, W. D., AND H. H. KRAMER, The genetic analysis of two chromosome interchanges in barley from F_2 data.

GERSH, EILEEN SUTTON, Influence of temperature on the expression of position effects in the scute-8 stock of *Drosophila melanogaster* and its relation to heterochromatization.

RUSSELL, ELIZABETH S., Analysis of pleiotropism of the W-locus in the mouse: Relationship between the effects of W and W^v substitution on hair pigmentation and on erythrocytes.

ROLLINS, W. C., S. W. MEAD, W. M. REGAN, AND P. W. GREGORY, The effect of inbreeding upon growth in Jersey cattle.

JANUARY, 1950

BEADLE, G. W., Rollins Adams Emerson.

CLAUSEN, R. E., and D. R. CAMERON, Inheritance in *Nicotiana tabacum*. XXIII. Duplicate factors for chlorophyll production.

WALTERS, MARTA SHERMAN, Spontaneous breakage and reunion of meiotic chromosomes in the hybrid *Bromus trinii*×*Bromus maritimus*.

WHITTINGHILL, MAURICE, Consequences of crossing over in oögonial cells.

GARDNER, ELDON J., and CHARLES M. WOOLF, The influence of high and low temperatures on the expression of tumorous head in *Drosophila melanogaster*.

OPATOWSKI, I., On the interpretation of the dose-frequency curve in radiogenetics.

MARKERT, CLEMENT L., The effects of genetic changes on tyrosinase activity in Glomerella.

STORMONT, CLYDE, Additional gene-controlled antigenic factors in the bovine erythrocyte.

Abstracts of Papers presented at the 1949 Meetings of the Genetics Society of America.

MARCH, 1950

KERR, WARWICK E., Genetic determination of castes in the genus Melipona.

MORGAN, DELBERT THOMAS, JR., A cytogenetic study of inversions in *Zea mays*.

BISHOP, CHARLES J., Differential X-ray sensitivity of Tradescantia chromosomes during the mitotic cycle.

HINTON, TAYLOR, A correlation of phenotypic changes and chromosomal rearrangements at the two ends of an inversion.

LINCOLN, RALPH E., and JOHN W. PORTER, Inheritance of *beta*-carotene in tomatoes.

DEMPSTER, EVERETT R., and I. MICHAEL LERNER, Heritability of threshold characters.

WAGNER, R. P., C. H. HADDOX, R. FUERST and W. S. STONE, The effect of irradiated medium, cyanide and peroxide, on the mutation rate in Neurospora.

NOVITSKI, E., The transfer of mutant genes from small inversions.

GECKLER, ROBERT P., Genetic changes induced by exposure to nitrogen mustard and their inheritance in *Paramecium aurelia*, Variety 4.

MAY, 1950

SLIZYNSKI, B. M., Partial breakage of salivary gland chromosomes.

DOBZHANSKY, TH. Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*.

GRIFFING, BRUCE. Analysis of quantitative gene action by constant parent regression and related techniques.

KHAMBANONDA, IAN, Quanitative inheritance of fruit size in red pepper (*Capsicum frutescens* L.).

PREER, JOHN R., Microscopically visible bodies in the cytoplasm of the "Killer" strains of *Paramecium aurelia*.

FOGLE, H. W., and T. M. CURRENCE, The inheritance of fruit weight and earliness in a tomato cross.

RAUT, CAROLINE, The effect of concentration of pantothenate on selection of a mutant for pantothenate synthesis on Saccharomyces cerevisiae.

SINGLE NUMBERS $1.25 ANNUAL SUBSCRIPTION $6.00

obtainable from

BROOKLYN BOTANIC GARDEN, BROOKLYN 25, NEW YORK